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411 ANNOUNCEMENTS

Is Immediate Chest Radiotherapy Obligatory for Any or All Patients With Limited-Stage Non-Small Cell Carcinoma of the Lung? Yes.[1]

James D. Cox,* Ritsuko Komaki, and Roger W. Byhardt[2]

External irradiation with the greatest technical sophistication available is indicated in all patients with inoperable non-small cell carcinoma of the lung confined to the primary site and to regional lymph nodes including the ipsilateral supraclavicular nodes. Irradiation with moderately high doses results in responses in 40%–65% of all patients. With irradiation to a level of at least 6000 rad in 30 fractions in 6 weeks, > 60% of patients never fail in the chest. Some of these patients become long-term, disease-free survivors. Until it is possible clearly to predict those patients who do not respond and thereby to justify an approach other than thoracic irradiation, it is necessary to offer radiation therapy to all of these patients. Since local control is far from satisfactory, one can certainly justify investigating new approaches, including further increases of the total dose, altered fractionation schemes, addition of radiosensitizers, and high linear energy transfer radiations. Since distant metastases frequently appear in spite of thorough pretreatment evaluations, one can also justify investigating the addition of systemic agents, both cytotoxic drugs and biologic response modifiers. Benefit from these systemic approaches may be completely masked, even in patients with disseminated disease, by failure to prevent complications and death due to the intrathoracic tumor. [Cancer Treat Rep 67:327–331, 1983]

The question for consideration concerns a very large number of patients in the United States. The American Cancer Society estimated 129,000 new patients with cancer of the lung in 1982. At least 20% of these patients would be expected to have small cell carcinoma (1). Of the remainder, 15%–20% will have resectable tumors, and approximately 50% will prove to have distant metastases. Nearly one-third of all patients with squamous cell carcinoma, adenocarcinoma, and large cell carcinoma will prove to have disease limited to the primary site with or without hilar, mediastinal, or ipsilateral supraclavicular lymph nodes, and with one or more criteria of unresectability (2). Thus, it can be estimated conservatively that about 30,000 new cases of this type will be seen in the coming year.

Which of these patients should receive thoracic irradiation as soon as the diagnosis is established? With full recognition of the limitations in our understanding of these types of bronchopulmonary cancer, the available data lead to the conclusion that *all patients with unresectable non-small cell carcinoma without metastases should receive immediate radiation therapy.*

It is conceivable that someday it may be possible to identify subsets of patients who could be treated by other means. It is certainly appropriate to pursue investigations that might lead to an increase in the effectiveness of thoracic irradiation (high linear energy transfer radiations, radiosensitizers, and altered fractionation) or might decrease the frequency of distant metastasis (biologic response modifiers and cytotoxic chemotherapeutic agents). However, at present nothing equals, let alone exceeds, the benefits of well-planned, fractionated thoracic irradiation to high total doses for patients with unresectable tumors confined to the primary site and regional lymph nodes.

The following discussion will address first the data which show the effectiveness of thoracic irradiation and thus justify this conclusion. Thereafter, some of the common arguments against immediate radiation therapy will be discussed.

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JUSTIFICATION FOR IMMEDIATE THORACIC IRRADIATION

Studies of failure patterns and causes of death show the importance of the intrathoracic tumor in determining survival.—Veterans Administration Lung Group (VALG) studies of clinical failure patterns by cell type, in which slides were reviewed and classified by a three-member panel of pathologists, showed distinct differences between squamous cell carcinoma and adenocarcinoma-large cell carcinoma; there were no clear differences between the latter two cell types (3). The dominant site of clinical failure after radiation therapy for patients with squamous cell carcinoma was local. For patients with adenocarcinoma and large cell carcinoma, a significantly smaller proportion of patients failed locally, and distant dissemination was more common. Similar results were found when assessing, by autopsies, the causes of death by cell type: squamous cell carcinoma less frequently had spread beyond the thorax at autopsy, whereas distant dissemination was found more frequently with adenocarcinoma and large cell carcinoma (4). Future studies of this type may show that improved local tumor control is associated with an increased frequency of distant metastasis. This has already been noted in studies of the Radiation Therapy Oncology Group (RTOG) (5). This does not diminish the importance of the local tumor as a major cause of death but rather indicates that the intrathoracic tumor must be overcome before any systemic approach can be expected to exert a beneficial effect.

Patients who respond to irradiation have a longer survival than those who fail to respond.—Data from VALG and from RTOG clearly show that complete responders have the best survival, partial responders an intermediate survival, and nonresponders the poorest survival experience (6,7). Between 40% and 65% of patients had complete or partial responses depending on the dose-fractionation regimen. If it were possible to predict which patients would respond and which would not, one could justify alternative or additional therapies. However, it is not yet possible to make such predictions. Neither the size of the primary tumor, the extent of regional lymph node involvement, nor the histopathologic type distinguish individual responders from nonresponders, although there is some correlation between high T and N stages and lower response rates (table 1). In the VALG studies (7) the response rate for squamous cell carcinoma was significantly greater than for the other cell types ($P = 0.03$). However, the RTOG found a greater response rate for patients with adenocarcinoma and large cell carcinoma than for squamous cell carcinoma (6). The median survival of the VALG patients with complete or partial responses was 50 weeks compared with 31 weeks for the patients who failed to respond. Although initial performance status (8) is known to be one of the most important prognostic factors for survival in patients with inoperable carcinoma of the lung (9), it did not influence the rate or duration of response of the thoracic tumor in the most recent VALG study (7). Since there are no identifiable criteria to predict consistently the nonresponders among patients with inoperable squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung, all of these patients should receive thoracic irradiation.

TABLE 1.—Unresectable squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung: response by stage of thoracic tumor*

Stage	No. of patients	Complete response (%)	Complete and partial response (%)
T1 N2	17	53	82
T2 N2	96	21	82
T3 N0	64	17	55
T3 N1	63	14	46
T3 N2	138	12	48

*From ref 5.

Radiotherapeutic control of the intrathoracic tumor improves short-term and long-term survival.—Retrospective studies at the Medical College of Wisconsin (MCW) showed that control of the tumor within the field of thoracic irradiation was associated with an improvement in survival (10,11). Median survival was improved slightly (10 vs 5.5 months), but the effect of local control was shown more impressively by survival beyond the median (fig 1). Retrospective studies (11,12) and prospective trials (5,6) have shown that total doses and dose-fractionation regimens are major determinants of local control. High total doses and continuous fractionation regimens with at least five fractions per week, without an interruption ("split-course"), are associated with the best local control (table 2). In addition, adequate coverage of the known and probable extensions of the tumor correlates significantly with improved survival (5).

Long-term, disease-free survival is possible with thoracic irradiation.—It seems obvious, but nonetheless deserves emphasis, that local control is essential for long-term, disease-free survival. A better understanding of the technical factors of thoracic irradiation which contribute to local control has resulted in improved survival. Of the 197 patients with inoperable carcinoma of the lung treated from 1971 through mid-1975 at MCW, 174 patients had squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (10). Ten patients (5.7%) lived at least 3 years, and eight were alive and well $\geq$ 5 years; patients died of intercurrent disease at 41 and 56 months, free of clinical evidence of cancer (13). A more consistent, aggressive approach to irradiation of patients with carcinoma of the lung began at the time of these 1975 studies: higher total doses were administered through multiple fields with individualized poured

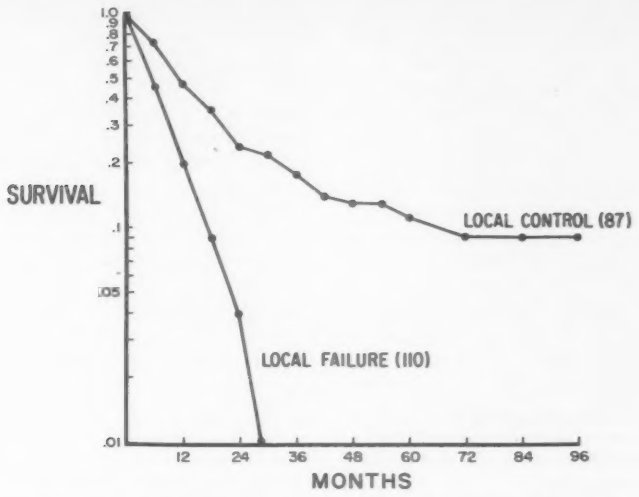

FIGURE 1.—Survival of patients with unresectable carcinoma of the lung, by control vs failure to control the tumor within the irradiated volume.

blocks, the entire mediastinum was consistently encompassed, and patients were treated at least five times per week. A review of the patients treated in the subsequent 3 years (14), mid-1975 through 1978, revealed 181 patients with localized but unresectable squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, of whom 29 (16%) lived a minimum of 3 years. There is no evidence that patient selection entered into the increased number of 3-year survivors. Indeed, many of the 3-year survivors in the more recent period had extensive lymph node involvement. Since 1978, further refinements of radiation therapy techniques have been made including a clearer understanding of the dose-fractionation regimens that increase local control rates (6,12), the importance of covering both the ipsilateral and contralateral hilar nodes and the entire mediastinum (5), more sophisticated treatment planning with simulation and computed tomography, and more detailed dosimetry; these developments can be expected further to improve local control and survival. More studies are needed in the following areas: (*a*) methods for increasing response rates and improving local control; (*b*) factors associated with response and local control with irradiation; and (*c*) factors associated with long-term as opposed to the short-term (median) survival.

CRITIQUE OF ARGUMENTS FOR WITHHOLDING THORACIC IRRADIATION

All patients die of distant metastases.—This commonly held belief is not supported by the literature. Attempts to evaluate causes of death at autopsy showed

TABLE 2.—Unresectable squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung: control within irradiated volume by dose-fractionation regimen

RTOG 73-01*		MCW 1971–1976†	
Regimen (Gy)	% controlled‡	Regimen (Gy)§	% controlled‡
40¶	42 (100)	< 40	12 (41)
40**	52 (100)	40–49	37 (19)
50¶	55 (91)	50–55	61 (41)
60¶	63 (84)	56–60	56 (39)
		> 60	70 (33)

*Prospective, randomized trial (ref 6).
†Retrospective study.
‡Values in parentheses = No. of patients.
§1.8–2.0 Gy/fraction, 5 fractions/wk (modified from ref 12).
¶2.0 Gy/fraction, 5 fractions/wk, consecutive wks.
**4.0 Gy/fraction, 5 fractions in 1 wk—2-wk "split" with no treatment—5 fractions in 1 wk.

that many patients died of complications of the intrathoracic tumor (15). A more recent study was based on 300 consecutive autopsies of patients from VALG protocols (4): only one-quarter of the patients with squamous cell carcinoma and approximately one-half of those with adenocarcinoma and large cell carcinoma had metastases beyond the thorax. Most of the remaining patients died of complications of the intrathoracic tumor. As more effective treatment for the thoracic tumor is developed, a greater proportion of patients should survive longer; they may have an increased risk of manifesting distant metastases. However, the demonstration that local control by irradiation significantly prolongs survival and the fact that a distinct subset of patients has long-term, disease-free survival show the importance of the thoracic tumor in determining outcome. Therefore, a careful search for metastases is warranted, but in the absence of disseminated disease, an aggressive approach to the local tumor will permit long-term survival for many patients.

A randomized study of radiotherapy versus placebo showed little benefit.—Reference is often made to an early VALG study in which some patients received thoracic irradiation while others received a placebo (16). The major problem with this study was that the radiation therapy in large part was inadequate: only 10% of the irradiated patients received cobalt 60 teletherapy or supervoltage irradiation; the remaining 90% of patients were treated with conventional x-rays. Cancer of the lung, like many deep-seated malignant tumors of viscera, showed little benefit from conventional x-irradiation. This study was also relatively unsatisfactory in that some patients with pleural effusion and peripheral metastases were still considered to have "limited" disease. Furthermore, detailed specifications were not made in regard to the volume that was to be irradiated or the dose-time relationships to be followed. Of course, modern treatment planning with simulators, detailed dosimetry, and custom blocks was not available. Is it necessary to repeat this study in an era of sophisticated radiation therapy? This might be conscionable for some investigators, but the following facts are sufficient reasons not to withhold thoracic irradiation: (*a*) patients die quickly from the local tumor if untreated, (*b*) control of the thoracic tumor greatly prolongs survival, and (*c*) there are long-term, disease-free survivors after radiation therapy.

Asymptomatic patients do not require any treatment.—The arguments for and against this statement (17,18) have not been based on data from asymptomatic patients. In the detailed study of prognostic factors from VALG patients reported by Stanley (9), only 1.8% of patients were truly asymptomatic, ie, had a performance status (PS) of 100. A much larger proportion of patients had symptoms but were able to carry on normal activities (PS, 80–90): 1671 of 5022 (33.3%) were in this group. VALG statistical reports (19,20) indicated that 1279 patients with disease limited to the thorax were entered in studies between 1958 and 1967; of 289 patients who received placebo and supportive care, 11 (4%) were alive at 2 years. Of 82 patients with a PS of 80–100 who received only placebo, none was alive at 5 years. By contrast, of the 197 patients who underwent irradiation for inoperable carcinoma of the lung between 1971 and 1975 at MCW (10,13), 92 (47%) had a PS of 80–100: the median survival for patients with local failure was 6 months and the longest survivor lived 27 months; when the thoracic tumor was controlled, the median survival was 17 months, and 22% were alive at 5 years. Fifteen of these patients had small cell carcinoma of the lung and would not currently be considered for treatment by thoracic irradiation alone. Of the remaining 77 patients, eight (10%) were alive and free of disease at 5 years, and two additional patients died of intercurrent diseases without any evidence of cancer during the fourth and fifth years of follow-up. This should be considered a baseline experience from which further improvements can be made, since there was insufficient information at that time to know the optimal volume or dose of irradiation, few of these patients were treated with custom blocks, detailed dosimetry was not routinely used, and computed tomography was not yet available.

Later treatment, when symptoms are present, is as good as immediate treatment.—Although a randomized study of this approach has not been reported, the rate of progression of cancer of the lung, even in the most favorable patients, makes it hard to defend postponing treatment. In the study of prognostic factors by Stanley (9), the most favorable patients (those with a PS of 100, disease confined to one hemithorax, and no weight loss) had an estimated median survival of only 72 weeks. Patients with limited disease and no weight loss, who had symptoms but were able to carry on normal activities with some effort (PS, 80), had a median survival of 48 weeks, and moderate weight loss (< 10% of body weight) dropped the median survival to 36 weeks. Since the development of any symptom is clearly associated with diminished survival, there is little to support deferring treatment until such symptoms occur. Independent of the symptomatic state, there is a decreased rate of response to irradiation with more advanced intrathoracic tumor (table 1). Coupled with the data that showed the intrathoracic tumor to be a major cause of death, the decreased ability to control more advanced T and N stages supports a plan of immediate thoracic irradiation for all patients without distant metastases.

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Is Immediate Radiation Therapy Indicated for Patients With Unresectable Non-Small Cell Lung Cancer? No.[1]

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Non-small cell lung cancer patients referred for "curative" radiation therapy constitute a heterogeneous group. They range from individuals with potentially resectable cancers who, for medical or other reasons, do not undergo surgery to patients with bulky primary tumors, with or without tumor extension into adjacent thoracic structures and with or without hilar and/or mediastinal lymph node involvement. Patient symptomatology may vary from a normal state of well-being to severely disabling symptoms such as weight loss, weakness, and fatigue. Depending on tumor histology, degree of differentiation, and tumor size, patients differ in probabilities of having subclinical metastatic disease. Finally, tumor volume doubling time may also show marked variability from one patient to another. Because of the many possible permutations and combinations of the above factors, it is not surprising that a wide range of survival times are reported in the lung cancer radiation therapy literature. Current radiation therapy studies using "best" dose-fractionation schedules report median survival times of < 1 year, 2-year survival probability of 10%–25%, and 5-year survival probability of approximately 5% (1–10). Does this degree of benefit from radiation therapy warrant immediate treatment of all patients with inoperable or unresectable non-small cell lung cancer, with disease clinically encompassable within a reasonable radiation therapy port? The counter interpretation of such survival results is that they largely reflect natural history of disease, the long survivors being prognostically favorable individuals with slowly growing tumors of limited metastatic potential. The resolution of this question obviously requires a randomized study comparing, in prognostically comparable patients (11–13), immediate radiation therapy versus no treatment or delayed treatment. Considering the worldwide availability of large numbers of patients with unresectable non-small cell lung cancer, the relative ease with which tumor shrinkage can be evaluated on chest roentgenogram, and the profusion of radiation therapy studies aimed at developing optimal dose-fractionation schedules, it is disappointing that only two randomized studies, to be discussed shortly, have addressed this question. Neither study convincingly demonstrated a therapeutic advantage for immediate radiation therapy.

A further consideration against immediate radiation therapy is that such a policy might impede development of effective combined-modality treatment programs. Chemotherapy results in non-small cell lung cancer are continually improving (14,15). As effective chemotherapy becomes available, combined-modality programs, utilizing chemotherapy, surgery, and radiation therapy, similar to those currently being evaluated in head and neck cancer (16), might significantly improve therapeutic results.

RANDOMIZED STUDIES

Neither of the two randomized studies conducted to date is sufficiently free of defects to allow an unequivocal conclusion as to the efficacy of immediate radiation therapy. The Veterans Administration Lung Cancer Group trial of radiation therapy (4000–5000 rad/4–5 weeks) versus placebo demonstrated only marginal benefit for radiation therapy. At 12 months, 18% of radiation-treated patients were alive, compared to 14% of placebo-treated patients. However, this study included patients with small cell lung cancer, used orthovoltage equipment, provided better supportive care to radiation-treated patients, and reported only 12-month survival (17,18).

The cooperative Oxford study evaluated immediate radiation therapy (≥ 4000 rad/13–14 fractions/28 days, orthovoltage or megavoltage equipment) versus delayed treatment when distressful symptoms developed (19). The mean survival was 8.4 months for the im-

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mediate treatment group and 8.3 months for the delayed treatment group, with 59% of the latter patients never receiving radiation therapy. Difficulty in evaluating these study results arises because of the inclusion of small cell lung cancer patients, because of the use of orthovoltage radiation therapy equipment, because no data are presented to indicate that the two patient groups were prognostically comparable, and because the study included only 70 patients with histologically confirmed non-small cell lung cancer.

Since definitive randomized studies are not available to answer questions as to the utility of immediate radiation therapy, the remainder of this paper will consider factors that determine the outcome of attempted curative radiation therapy. It will be pointed out that only a relatively small percentage of radiation-treated patients can be expected to have disease confined within the radiation therapy port. From consideration of the growth kinetics of lung cancer it will be suggested that some long surviving patients might have lived as long with or without radiation therapy. Finally, it will be indicated that clinically achievable radiation therapy doses produce a complete response in only a minority of treated patients.

INTRAPULMONARY GROWTH OF LUNG CANCER

Because radiation therapy kills only tumor cells within the radiation port, it is important to consider the intrapulmonary spread of lung cancer (20,21). It is clear that both centrally and peripherally arising lung neoplasms may extend to involve substantial amounts of adjacent lung tissue, beyond that which can safely be incorporated in a radiation port. From their origin in bronchial mucosa, centrally arising lung cancers may extend by endobronchial or by transbronchial growth. Endobronchial growth, via submucosal lymphatics, may produce mucosal tumors remote from the site of tumor origin. Generally, this type of extension occurs centripetally (in a medial or central direction) and would likely be within a radiation port that includes the primary lesion and mediastinal lymph nodes. Centrally arising tumors may also grow through the bronchial wall reaching peribronchial lymphatic vessels and connective tissue. Tumor cell flow within these lymphatics is initially central (centripetal) in direction. As lymphatics become obstructed by tumor, however, lymph flow may be reversed so that cancer cells travel peripherally (centrifugally). Similarly, tumor growth in peribronchial connective tissue may be either centripetal or centrifugal. Since the lateral radiation port margin generally extends only about 2 cm beyond the primary lesion, it is likely, especially in individuals with involved hilar and mediastinal lymph nodes, that transbronchial tumor extension and growth account for some intrathoracic failures outside of the radiation port.

Peripheral lung cancers arise from distal bronchi and bronchioles. As the lesions grow they displace normal lung tissue (hilic growth), or tumor cells may line and fill alveoli similar to a pneumonic infiltrate (lepidic growth). As with centrally arising tumors, these neoplasms also invade peribronchial lymphatics and connective tissue. While lymphatic flow is usually centripetal with lymphatic obstruction, especially with hilar lymph node involvement or with lymphatic obstruction by the primary tumor itself, retrograde lymphatic flow occurs with extension of tumor cells into the lymphatic network of the visceral pleura. The pleural lymphatic network is extensive; it crosses lobar fissures and may also extend to the parietal pleura and chest wall through lymphatic channels that develop within adhesions between visceral and parietal pleura. The widespread patterns of intrathoracic spread of peripherally arising neoplasms, similar to that of centrally arising tumors, is likely to result in tumor cells being located outside the radiation therapy port.

SYSTEMIC SPREAD OF LUNG CANCER

Presence of occult metastatic disease is an accepted major cause of failure of curative radiation therapy. Matthews and co-workers (22,23) evaluated a series of 254 patients who died of non-cancer-related causes within 30 days of a "curative" surgical resection. Persistent disease was found in 53 of 171 patients with epidermoid cancer (35%), with incidence equally divided between patients with regional-only residual disease and those with distant metastases. Sixteen of 34 patients (47%) with adenocarcinoma had persistent disease, with distant metastases present in 13 of the 16, while four of 25 patients (16%) with large cell anaplastic carcinoma had persistent disease, with distant metastases present in all. Matthews and co-workers (23) further classified a subgroup of the above patients into T and N categories (24). It was found that the frequency of persistent disease increased with worsening T status. For patients with epidermoid lung cancer, 14% with T_1 lesions had persistent disease as compared to 35% and 77% with T_2 and T_3 tumors, respectively. For adenocarcinoma patients, 27%, 40%, and 55% with T_1, T_2, and T_3 tumors, respectively, had persistent disease. Since the frequency of hilar lymph node involvement was found to increase progressively as primary tumors worsened from T_1 to T_3, it was also evident that individuals with positive hilar nodes were more likely to have persistent disease than were individuals with negative nodes.

The above data were compiled from patients whose tumors were thought to be surgically resectable. Data on the frequency of systemic metastases in the types of patients referred for radiation therapy can perhaps be better obtained from patterns of failure of radiation therapy patients. Because median survival times of pa-

tients receiving radiation therapy are < 1 year (1–10), it is highly likely (to be discussed) that metastases detected during or after radiation therapy were also present at the start of therapy.

Failure patterns of patients entered into a Veterans Administration Lung Group radiation therapy study were reported by Stanley et al (25) and by Cox and Yesner (26). Of patients with squamous cell lung cancer, 23% died of causes believed to be unrelated to cancer, 28% died of local failure alone, and 49% died of distant failure alone or with accompanying local failure. For patients with adenocarcinoma, corresponding figures were 25% of deaths from non-cancer-related causes, 5% from local failure alone, and 70% from distant failure alone or with accompanying local failure. The Radiation Therapy Oncology Group, using 5000- or 6000-rad continuous radiation therapy, reported local failure alone in 15% and 11%, respectively, and distant failure alone or with accompanying local failure in 57% and 55%, respectively (27).

Both of these trials, largely reporting clinical failure patterns, almost certainly underestimate the frequency of distant metastases in irradiated patients with lung cancer. Nevertheless, even in these studies 50%–70% of patients or more, depending on histology, were likely to have had systemic metastases at the time that treatment was initiated.

TUMOR GROWTH RATE

An additional factor that determines survival of a patient with lung cancer is tumor growth rate. The model of Collins et al (34) indicates that 30–35 doublings take place before a tumor reaches a clinically diagnosable size (1–3 cm). An additional five to ten doublings produce a lethal tumor burden. At diagnosis, therefore, all patients are relatively late in the natural history of disease, and it is likely that systemic metastases have already occurred if they are going to occur. Tumor growth rate (measured clinically as tumor doubling time) and tumor size at presentation are the two major factors that determine patient survival (35). Straus (36) and Geddes (35) have summarized the world literature on volume doubling times of the non-small cell lung cancer histologies. For epidermoid cancer and large cell anaplastic carcinoma, the mean doubling time is approximately 100 days (range, 30–380), while the mean doubling time for adenocarcinoma is approximately 180 days (range, 30–380). Based on the above considerations the fortunate patient who presents with a 1-cm tumor with an average doubling time of 300 days can expect to survive beyond 5 years without any treatment. Clinical verification of these predications can be derived from long-duration follow-up of surgical results. In one such series of 963 patients who survived surgical resection, there were a total of 869 deaths. Of these deaths, 92 occurred between 5 and 10 years after surgery, and 63 occurred beyond 10 years. From death certificate review, it was determined that 53% of the deaths in the former group and 39% of the deaths in the latter group were due to bronchogenic carcinoma. While second primary lung cancers might account for some of these late deaths, it is evident that some fraction of patients had residual, slowly growing carcinoma (37).

CYTOTOXIC ACTIVITY OF RADIATION THERAPY

It is difficult to precisely determine the cytotoxic capability of radiation therapy. Obviously, tumor-related factors such as tumor size and tumor location (6,28) and host-related factors such as performance status, weight loss, and pulmonary function (11–13) affect treatment results. Patient selection might, therefore, account for some of the wide disparity of reports concerning local control by radiation therapy. Two autopsy studies indicate that local control was achieved in 21%–38% of cases (29,30), while three preoperative radiation therapy studies reported that local control of the primary lesion was achieved in 27%–50% of cases (31–33). A recently completed clinical study by the Radiation Therapy Oncology Group indicated a complete response rate of 24% in patients treated with 5000-rad continuous radiation and 21% in patients treated with 6000-rad continuous radiation (6). However, three other recently completed studies report complete response rates < 10% (7–9). The low complete response rates achieved in these latter studies are discouraging.

In summary, multiple factors prevent achievement of "curative" radiation therapy in patients with unresectable non-small cell lung cancer. Therefore, it does not seem reasonable to recommend that this treatment be offered immediately to all such patients. Since the biology of lung cancer growth results in regional and systemic disease outside of the radiation port in a significant number of patients, it seems worthwhile to expend a major effort in developing effective chemotherapy regimens for these patients. Since, in the future, combined chemotherapy and radiation therapy will probably represent optimal treatment for this patient group, it is also necessary to find ways of minimizing the enhanced pulmonary toxicity commonly noted with combined-modality therapy (38,39).

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Phase II Clinical Evaluation of 4′-Epi-doxorubicin[1]

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4′-Epi-doxorubicin (4′e-Dx) was used as a single agent in a broad phase II study involving a variety of advanced metastatic tumors. A total of 115 patients were treated, of whom 93 were evaluable. The dosage was 75 mg/m²/cycle repeated every 21 days, with a maximal cumulative dose of 550 mg/m². Nine responses were achieved in a total of 28 patients with breast cancer (32%). The response rate was 54% in breast cancer patients receiving 4′e-Dx as first-line treatment, 25% in patients previously given chemotherapy without doxorubicin, and 11% in patients previously given chemotherapy with doxorubicin. Some therapeutic activity was also detected in endometrial carcinoma, epidermoid carcinoma of the cervix, non-Hodgkin's lymphoma, melanoma, and skin epidermoid tumor, but the number of patients entered in the study with these various tumors was too limited to allow any statement on efficacy of 4′e-Dx. No treatment schedule with 4′e-Dx had to be interrupted because of toxicity. In a total of 373 evaluable cycles, only three acute reversible ECG abnormalities were recorded. In 16 of 109 evaluable patients there was some minor or moderate alteration of one or more of the three major blood cell parameters following 4′e-Dx therapy, requiring a postponement of the next cycle by ≤ 10 days. [Cancer Treat Rep 67:337–341, 1983]

4′-Epi-doxorubicin (4′e-Dx) is a derivative of doxorubicin (Dx) obtained by epimerization of the hydroxyl group in position 4′ in the sugar moiety; it is synthesized by Farmitalia Carlo Erba Research Laboratories. Its therapeutic index, which was found to exceed that of Dx in various experimental tumor models, and its lower cardiotoxicity in the rat and rabbit (1,2) have led to its selection for clinical evaluation. In a more recent paper, Dantchev et al (3) showed that in the golden hamster model, 4′e-Dx and Dx had the same cardiotoxic effect.

Pharmacokinetic studies conducted initially in animals (4) and subsequently in man (4–7) demonstrated that there are significant differences between the two drugs regarding distribution, metabolism, and excretion. Whereas both drugs were characterized by a large volume of distribution indicating a high tissue-plasma ratio, 4′e-Dx appeared to be metabolized and excreted faster. The terminal half-life in man was about 30 hours for 4′e-Dx versus 43 hours for Dx. These differences in pharmacokinetic behavior may account for the lower acute and chronic toxicity of 4′e-Dx.

In a phase I clinical trial carried out by Bonfante et al (8) using doses of 50, 60, 75, and 90 mg/m² of 4′e-Dx given by iv bolus every 3 weeks, the toxicity symptoms recorded were of the same type as, but of lower incidence and intensity than, those observed with Dx at the same doses. Myelosuppression was the major dose-limiting toxic effect, but the study failed to demonstrate a dose-effect relationship as the doses were escalated to 90 mg/m². On the other hand, a therapeutic activity similar to that of Dx was detected for 4′e-Dx, with a low degree of toxicity, at doses of 75–90 mg/m².

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As the early results of Bonfante et al began to support a therapeutic index that was higher for 4′e-Dx than for Dx, the Clinical Screening Group of the European Organization for Research on Treatment of Cancer (EORTC) initiated a broad phase II clinical study of 4′e-Dx in advanced solid tumors. The group considered that the clinical data available on the efficacy and toxicity of 4′e-Dx were not sufficient to warrant the initiation of a disease-oriented phase II study.

MATERIALS AND METHODS

This study was confined to patients with histologically proven advanced solid tumors or lymphosarcomas. All patients had been found to be resistant to conventional therapy, with the exception of ten patients with advanced breast cancer who received 4′e-Dx as first-line chemotherapy.

To be eligible for study, the patients had to fulfill the following conditions: age, 15–70 years; performance status (Karnofsky scale) $\geq$ 60%; documented and measurable or evaluable lesions; no prior radiation therapy or chemotherapy within 4 weeks; wbc count $>$ 3000/mm^3; platelet count $\geq$ 100,000/mm^3; normal hepatic and renal functions; and estimated survival time $>$ 2 months. Patients were not eligible for study if they met any of the following criteria: prior Dx cumulative dose $>$ 250 mg/m^2; other concurrent malignant disease except in situ carcinoma of the cervix and/or correctly treated skin carcinoma; current uncontrolled infection; central nervous system metastases; congestive heart failure; or cardiac arrhythmia, bilateral bundle branch block, or a history of myocardial infarction.

4′e-Dx was supplied by Farmitalia Carlo Erba Research Laboratories as a sterile red lyophilized powder in 10- and 50-mg, rubber disc-capped vials. The drug was reconstituted with sterile water and administered by iv injection through the side arm of a freely flowing iv infusion line. 4′e-Dx was given as a single 75-mg/m^2 iv dose, repeated at 21-day intervals. At least three cycles of treatment were given; if major disease progression occurred, treatment with 4′e-Dx could be stopped after the second cycle. Treatment was stopped in any case when a cumulative dose of 550 mg/m^2 of 4′e-Dx was reached.

Therapeutic activity was evaluated according to World Health Organization criteria (9). Brain metastases, ascites, and pleural effusion were not considered criteria for evaluation. Drug toxicity was evaluated according to criteria defined by Chauvergne (10). For the sake of consistency in the recording and reporting of results, the trial coordinator visited each trial center and checked every patient file with the clinical investigator in charge.

RESULTS

Therapeutic Response

The clinical trial with 4′e-Dx involved 115 patients, of whom 93 were evaluable. Twenty-two patients were not evaluable for therapeutic activity because of early death or protocol violation (nonevaluable disease; stopping of treatment after one cycle, independently of severe toxicity or progressive disease; and loss to follow-up before evaluation). The average age was 52 years (range, 15–70). There were 37 men and 56 women (sex ratio, 0.40). The median Karnofsky index was 70%; 55 patients (59%) had received prior chemotherapy, and 23 patients (25%) had received prior chemotherapy that included Dx.

Therapeutic responses obtained in this study are given in table 1. With a total of 32 patients with breast cancer,

TABLE 1.—Therapeutic activity*

Site or type of primary tumor	No. of patients				
	Total	CR	PR	NC	PD
Breast	28	1	8	18	1
Head and neck	12	—	—	12	—
Genitourinary	11	—	—	9	2
Gastrointestinal	11	—	—	5	6
Endometrium	6	—	1	5	—
Cervix	5	—	1	3	1
Sarcoma	6	—	—	4	2
Ovary	3	—	—	3	—
Lung	3	—	—	3	—
Melanoma	3	—	1	1	1
Skin, epidermoid	3	—	1	2	—
Non-Hodgkin's lymphoma	2	—	1	1	—
Total	93	1	13	66	13

*CR = complete response; PR = partial response; NC = no change; and PD = progressive disease.

TABLE 2.—Breast cancer: characteristics of responding patients

Patient No.	Prior Dx (mg/m²)	4′e-Dx cumulative dose (mg/m²)	Duration of response (wks)	Reason for stopping treatment
1	—	640	28+	Maximal allowed dose exceeded
2	—	525	21+	Maximal dose reached
3	—	375	15+	Response > 50%; no dose-limiting toxicity, but nonavailability of drug
4	—	525	21+	Maximal dose reached
5	—	375	15+	Response > 50%; no dose-limiting toxicity, but misinterpretation of protocol requirements
6	—	300	19+	Response > 80%, but complementary radiotherapy started
7	91	450	26	Development of cerebral metastases
8	—	825	33+	Response > 50%, but maximal cumulative dose exceeded
9	—	650	33+	CR, but maximal cumulative dose exceeded

28 of whom were judged evaluable, breast is the only cancer site for which the number of cases was high enough to allow statistical evaluation of a 20% activity with a β risk of 5%. With nine responses among 28 patients, the overall therapeutic activity of 4′e-Dx amounts to 32%. The only complete response obtained was in a patient who had breast cancer with pulmonary and soft tissue metastases (table 2). The time to complete response in soft tissue was 12 weeks, whereas the clearing of the pulmonary lesions on x-ray required 18 weeks of treatment. The median time to maximal response was 19 weeks (range, 6–29). Soft tissue and lymph node metastases were most sensitive to 4′e-Dx, with 16 responses (41%), of which five (13%) were complete (table 3). Pulmonary metastases, with a 33% response, also demonstrated a high sensitivity to 4′e-Dx treatment. The response rate to 4′e-Dx was clearly related to prior chemotherapy (table 4). 4′e-Dx used as first-line treatment induced a response rate of 54% in a total of 11 patients, with six objective responses (one of which was complete); when it was used as second-line treatment, the rate dropped to 25% after chemotherapy without Dx and 11% after chemotherapy with Dx. The latter finding is based on too few patients to determine whether there exists any cross-resistance between Dx and 4′e-Dx.

Some therapeutic activity was also detected in endometrial carcinoma (liver and lung metastases), epidermoid carcinoma of the cervix (pulmonary metastases), non-Hodgkin's lymphoma (diffuse histiocytic, spleen), melanoma (soft tissue site), and skin epidermoid tumors. However, for all these sites the number of patients entered into the study was too small to allow any statement regarding efficacy of 4′e-Dx.

Toxicity

Of 115 patients entered in the study, 109 were evaluable for toxic effects. The overall toxicity of 4′e-Dx was moderate under the conditions of this study (table

TABLE 3.—Therapeutic activity in breast cancer: responses according to tumor site

Tumor site	No. of patients					
	Total	CR	PR	CR + PR	NC	PD
Primary tumor	11	1	3	4 (36%)	7	0
Skin	17	3	5	8 (47%)	9	0
Node	11	1	3	4 (36%)	7	0
Bone	3	0	0	0	2	1
Lung	12	1	3	4 (33%)	8	0
Liver	1	0	0	0	1	0

TABLE 4.—Therapeutic activity in breast cancer: responses according to prior treatment

	No. of patients			
	Total	CR	PR	CR + PR
Prior chemotherapy				
With Dx	9	—	1	1 (11%)
Without Dx	8	—	2	2 (25%)
No prior chemotherapy	11	1	5	6 (54%)

5). One treatment had to be stopped for severe asthenia (IT 3 rating), with digestive intolerance after one course of treatment. There was no case of lethal toxicity (IT 4).

In 16 of 109 evaluable patients there was some alteration (IT 1 or IT 2) of one or more of the three major blood cell parameters following treatment. The next course of treatment had to be postponed by a maximum of 10 days to allow blood cell alterations to correct themselves. Leukopenia-neutropenia with a nadir of 1000 granulocytes/mm^3 was noted in six cases and/or thrombocytopenia with a nadir of 75,000 platelets/mm^3 was noted in three cases at blood check on Day 20.

The incidence of nausea and/or vomiting and of asthenia (accompanied by moderate fever in some cases) was 40%, but their intensity (IT 1–IT 2) did not require a modification in the treatment schedule. Alopecia was seen in 68% of cases.

Three cases of acute reversible ECG abnormalities were recorded in a total of 373 evaluable cycles, as follows: (*a*) one episode of nocturnal tachycardia-arrhythmia with ST wave alteration in a female patient who had received a cumulative 4′e-Dx dose of 225 mg/m^2; (*b*) one case of transitory and reversible reduction (< 20%) in the amplitude of the QRS complex following the first injection of 4′e-Dx in a patient who had received prior chemotherapy with a cumulative Dx dose of 150 mg/m^2; and (*c*) one case of reduction (> 30%) in the amplitude of the QRS complex in a patient who had received a cumulative 4′e-Dx dose of 150 mg/m^2.

The average cumulative dose of 4′e-Dx administered in this study was 264 mg/m^2. If prior total cumulative doses of Dx are added to the cumulative doses of 4′e-Dx, the average cumulative dose of anthracycline administered amounts to 283 mg/m^2. This calculation includes eight patients who received a > 500-mg/m^2 cumulative dose of 4′e-Dx. Eleven patients had received prior ^{60}Co radiotherapy in the left internal mammary area, at an average dose of 48.7 Gy (range, 45–50). In those patients, the average cumulative dose of 4′e-Dx had been 343 mg/m^2 and the average cumulative dose of anthracyclines (4′e-Dx + Dx) had been 378 mg/m^2.

There was no case of congestive heart failure, and cardiotoxicity was not a reason for interruption of 4′e-Dx treatment in any of the patients.

DISCUSSION

When used as a single drug at a dose of 75 mg/m^2 every 3 weeks, 4′e-Dx led to objective regression of breast cancer metastases, with minimal to moderate conventional toxicity. The results obtained in this study are consistent with those reported earlier by other investigators (11–13).

The acute toxicity of 4′e-Dx in this study was minimal to moderate. Again, this conclusion is consistent with that of the earlier clinical studies (11–13). Comparative studies with Dx used at equal and/or equitoxic doses should be conducted for a full evaluation of the new analog.

TABLE 5.—Toxic symptoms* (109 patients)

Toxic effect	IT 0	IT 1	IT 2	IT 3	IT 4
Nausea/vomiting	65	34	9	1	—
Stomatitis	107	2	—	—	—
Asthenia-fever	70	29	9	1	—
Alopecia†	24	52	—	—	—
Leukopenia	97	3	9	—	—
Thrombocytopenia	103	1	5	—	—
Anemia	104	5	—	—	—
Cardiac	106	3	—	—	—
Renal or hepatic	109	—	—	—	—

*Rated according to criteria defined by Chauvergne (ref 10).

†33 patients are not accounted for because they had previously experienced at least some hair loss; they should thus be rated IT 9 for alopecia.

The conditions set in the protocol for this study did not allow for an evaluation of the cardiotoxicity of 4′e-Dx. Even in the case of objective response, 4′e-Dx therapy was to be stopped when the cumulative dose of 550 mg/m^2 was reached. Besides, cardiac surveillance included in most instances only clinical evaluation and ECG. Interestingly, in one case of protocol violation where a cumulative dose of 825 mg/m^2 of 4′e-Dx was administered over an 8-month period, no modification in the results of radionuclide angiography ejection fraction was observed between baseline and end of treatment values.

Since the studies conducted in various animal models (1,3,14,15) suggest that the cardiotoxicity of 4′e-Dx may be ≥ 20% lower than that of Dx, further clinical studies involving careful cardiac monitoring (by echography and/or radionuclide angiography) seem advisable; they should be designed to demonstrate whether 4′e-Dx may be administered beyond the 550-mg/m^2 limit in order to prolong the duration of anthracycline therapy in responsive patients.

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Cardiac Evaluation of Mitoxantrone[1,2]

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Mitoxantrone is a synthetic anthraquinone that was developed through the doxorubicin analog program in hopes of retaining anticancer activity with less cardiotoxicity. This study evaluated 18 patients receiving mitoxantrone with serial noninvasive tests of left ventricular function and with endomyocardial biopsy. The echocardiograms and systolic time intervals demonstrated a trend to deterioration that did not achieve statistical significance. However, the nuclear angiographic ejection fraction significantly decreased from 61% ± 6% ($\bar{x}$ ± SD) at baseline to 58% ± 5% ($P < 0.05$) after 48 mg/m² of mitoxantrone. The endomyocardial biopsies revealed tubular swelling, degeneration of mitochondria, minimal chromatin clumping, and myofibrillar lysis. This study has revealed mild but definite impairment of cardiac function and mild changes of myocardial morphology during mitoxantrone therapy. Although mitoxantrone is an effective chemotherapeutic agent, a direct comparison of mitoxantrone with doxorubicin is necessary to compare relative therapeutic to cardiotoxic ratios. [Cancer Treat Rep 67:343–350, 1983]

Mitoxantrone is a synthetic anthraquinone developed through a search for agents retaining the anticancer activity of this class of compounds without the cardiotoxicity seen with doxorubicin. Mitoxantrone, like doxorubicin, is a DNA-reactive agent that induces frequent DNA strand breaks (1,2) and inhibition of DNA and RNA synthesis (3) in cultured cells. Mitoxantrone has antitumor activity similar in degree and breadth to doxorubicin in animal tumor models (4). Early clinical trials suggest that mitoxantrone may be useful in several human cancers, with impressive activity in breast cancer (5,6).

The potential for cardiotoxicity of mitoxantrone is unclear. Myocardial changes in rats, beagles, and monkeys during preclinical toxicity evaluation were limited to hemorrhage (in animals receiving lethal doses) and minimal vacuolation of sarcoplasmic reticulum (3). Several patients receiving mitoxantrone in phase I and early phase II clinical trials have developed congestive failure, although most have had pre-existing heart disease, prior doxorubicin therapy, and/or mediastinal irradiation. The incidence and severity of myocardial injury induced by mitoxantrone are presently unknown. The present study was designed to evaluate functional and morphologic changes in the human myocardium following mitoxantrone therapy.

METHODS

Eighteen patients (14 female, four male) were entered in the study. The average age of the entire group was 58 ± 12 years ($\bar{x}$ ± SD). The tumors for which these patients were to be treated included breast (eight patients), lung (two), leiomyosarcoma (two), kidney (two), lymphoma (one), cervix (one), esophagus (one), and leukemia (one). Eleven of the patients (Group 1) had received prior cardiotoxic therapy including doxorubicin in eight (dose, 345 ± 94 mg/m²; $\bar{x}$ ± SD), mediastinal radiation in five (dose, 4000 ± 2200 rads), and aclarubicin in one (dose, 380 mg/m²). The seven patients who had not received cardiotoxic therapy comprised Group 2. These studies were approved by the Human Re-

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search Committee at our hospital. Written informed consent was obtained from each patient prior to study.

Each patient had noninvasive testing prior to beginning mitoxantrone therapy and these tests were repeated prior to each dose of mitoxantrone. Mitoxantrone was administered as a single iv bolus at doses of 10–20 mg/m^2. The noninvasive cardiac function tests used to follow these patients were the echocardiogram and systolic time intervals in all patients. Resting nuclear angiography was also obtained whenever possible. During the study period, the only change in medications was the adjustment of mitoxantrone dose to achieve a desired level of myelosuppression.

Echocardiograms were performed with a Unirad Series C Echoscope and an Electronics for Medicine VR6 recorder or an SKF Echoline 20A echocardiograph with an Irex recorder. A single echocardiographic technician performed the echocardiograms. Blinded echocardiographic interpretation was performed in accordance with the recommendations of the American Society of Echocardiography (7). The % ΔD was used as the echocardiographic determinant of left ventricular function and was derived from the formula:

$$\%\Delta D = \frac{\text{end diastolic diameter} - \text{end systolic diameter}}{\text{end diastolic diameter}} \times 100.$$

Systolic time intervals were obtained with an Electronics for Medicine VR6 or a Smith, Kline, Echoline 20A unit utilizing the specifications previously outlined (8). The pre-ejection period (PEP) to left ventricular ejection time (LVET) was used as the measure of left ventricular performance. A single technician performed these tests and recorded the data. The reproducibility of these techniques in the outpatient setting has been previously demonstrated by our laboratory (9).

Resting nuclear angiography was performed by injecting 5.5 mg of stannous pyrophosphate iv. Following a 20-minute delay, 10 ml of blood was drawn for in vitro tagging with 25 mCi of technetium 99m pertechnetate. Imaging was performed in a modified LAO projection using a mobile Anger camera (GE Med III) with a high sensitivity parallel hole collimater. The data acquired were transmitted to a Medical Data Systems A^2 40,000 multiterminal computer system. The data were spatially and temporally filtered using Fourier filtering techniques to produce images with a high signal to noise ratio and good anatomic resolution. Background subtraction and construction of time-activity curves were performed in a standard fashion. The left ventricular ejection fraction (EF) was then calculated.

Five patients from Group 1 and two patients from Group 2 had endomyocardial biopsies. Tissue samples, 1–2 mm^3 in size, were removed from the right side of the interventricular septum by endomyocardial biopsy. Samples were placed in cold 2% glutaraldehyde, washed in 0.1 M phosphate buffer (pH 7.4), and postfixed with 1% osmium tetroxide. After ethanol dehydration, samples were embedded in epoxy resin for sectioning. Ultrathin plastic sections were cut with an LKB ultramicrotome, mounted on copper grids, and stained with uranyl acetate and lead citrate. An Hitachi Perkin Elmer HU12 electron microscope was used to observe tissue samples.

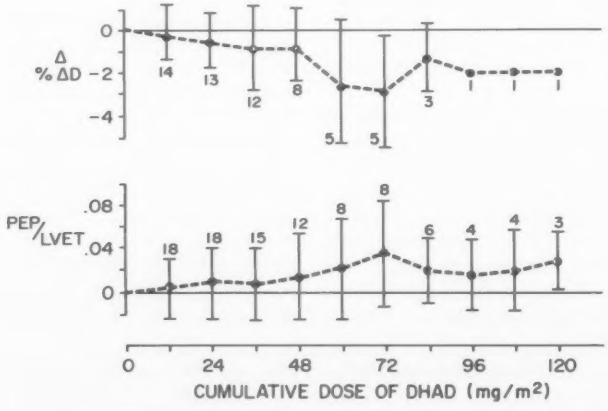

FIGURE 1.—Cumulative dose of mitoxantrone (DHAD) is shown on the horizontal axis. Top panel demonstrates change in echocardiographic %ΔD from baseline. Each data point represents $\bar{x} \pm$ SD; No. of patients studied at each point is shown. Bottom panel demonstrates change from baseline in systolic time intervals (PEP/LVET ratio). No change in either panel is significant.

Statistical Analysis

Serial noninvasive tests were evaluated by one-way analysis of variance. The Group 1 and 2 data were compared by the two-way analysis of variance.

RESULTS

One of the 18 patients developed congestive heart failure during the course of this study. Some changes of cardiac function and myocardial morphology did occur in the group as a whole. The baseline systolic time intervals, PEP/LVET, for all patients were 0.39 ± 0.06 ($\bar{x}$ ± SD). The baseline PEP/LVET for the previously treated (with doxorubicin, aclarubicin, or chest irradiation) Group 1 patients was 0.41 ± 0.07, and for Group 2 patients it was 0.37 ± 0.06. The change in the PEP/LVET for all patients from baseline is shown in figure 1. The change from baseline was not significant at any dose.

The baseline echocardiographic %ΔD for the 14 patients with technically satisfactory echocardiograms was 32% ± 3%, with no difference between Group 1 (32% ± 1%) and Group 2 (33% ± 4%). The change in the %ΔD for all patients from baseline is shown in figure 1. The change was not significant at any dose.

The change in the EF of the nuclear angiograms is shown at baseline and after 48 mg/m² in seven patients (four from Group 1, three from Group 3) and after 96 mg/m² in two patients (one from each group) in figure 2. The change from baseline was significant ($P < 0.05$) at 48 mg/m².

The changes of the cardiac noninvasive tests in Group 1 (previous cardiotoxic therapy plus mitoxantrone) were contrasted to the changes in Group 2 (mitoxantrone only). There were no differences between the groups in the PEP/LVET, %ΔD, or EF.

An endomyocardial biopsy procedure was performed on a patient who had received no prior cardiotoxic therapy but had received 32 mg/m² of mitoxantrone. His biopsy was totally normal by light and electron microscopy.

Another patient (a 59-year-old woman with a normal cardiac history and physical examination, who had not received prior cardiotoxic therapy) had an endomyocardial biopsy after 120 mg/m² of mitoxantrone. Her myocardium was normal by light microscopy, but electron microscopy demonstrated minimal changes of tubular structures, mild degeneration of mitochondria, increased amounts of lipofuscin pigment, and mild chromatin clumping (fig 3). This patient had achieved a complete remission of breast cancer and therefore received an additional 88 mg/m² of mitoxantrone (total cumulative dose, 208 mg/m²), at which time treatment was stopped. Within 1 month she developed heart failure (PEP/LVET, 0.48; %ΔD, 14%; angiographic EF, 29%).

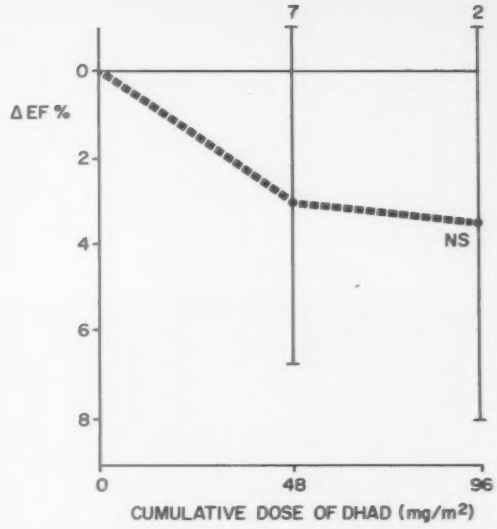

FIGURE 2.—Cumulative dose of mitoxantrone (DHAD) is shown on the horizontal axis, and change from baseline in nuclear angiographic EF (ΔEF) is shown on the vertical axis. 7 patients observed at baseline had an EF of 61% ± 6%. This decreased to 58% ± 5% ($P < 0.05$) at 48 mg/m². Two patients had a 3rd EF at 96 mg/m².

The endomyocardial biopsy was repeated (fig 4). Myofibrillar lysis was now present in occasional cells, and the swelling of mitochondria and tubular structures was more severe.

A third patient had two endomyocardial biopsy procedures. The first biopsy was after 80 mg/m² of doxorubicin (left panel, fig 5). This tissue demonstrated some swelling of T tubules and mitochondria. His second biopsy was after 122 mg/m² of mitoxantrone. The myocardial morphologic alterations were not significantly different from those seen in the first biopsy (right panel, fig 5).

For a fourth patient who had received 4675 rads to her chest for breast cancer and then 130 mg/m² of mitoxantrone, cardiac function tests were normal, and the endomyocardial biopsy (fig 6) demonstrated only mild tubular changes with normal myofibrils. The nucleolus, shown on the right in figure 6, was contracted.

Another three patients had each received doxorubicin in total cumulative doses of 370, 445, and 460 mg/m². At the time of endomyocardial biopsy, each patient had also received mitoxantrone in cumulative doses of 32, 100, and 120 mg/m², respectively. These biopsies were similar to those described above. The most impressive change was the swelling of tubular structures. Mitochondria were minimally damaged and nuclear morphology was consistent with the alterations reported from doxorubicin alone (10).

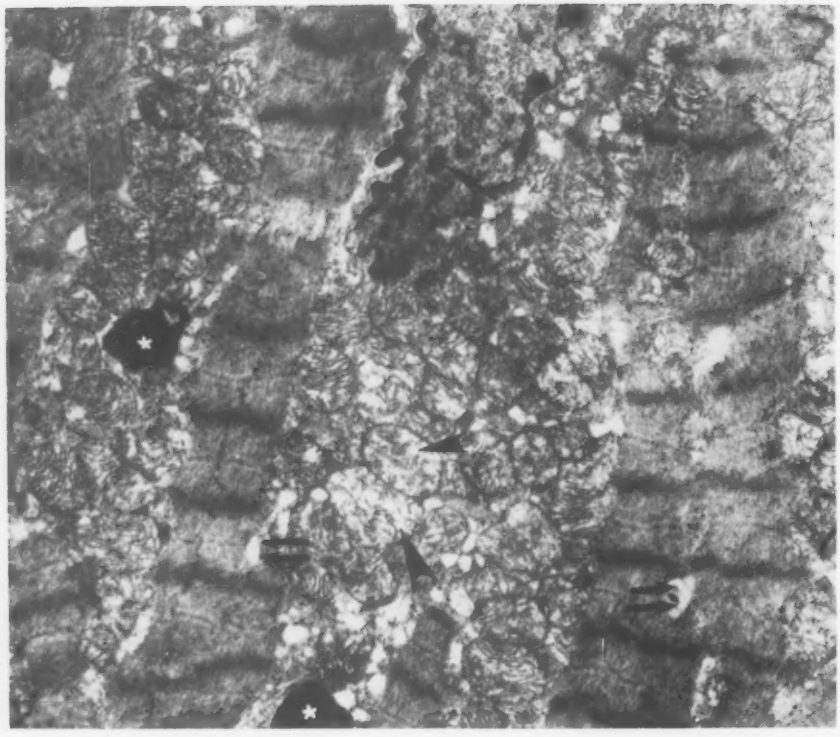

FIGURE 3.—Electron micrograph (× 15,000) from a patient who had received 120 mg/m^2 of mitoxantrone but no other cardiotoxic therapy reveals mild chromatin clumping (single arrow), dilatation of tubular structures (double arrows), and mild mitochondrial degeneration (arrowheads). Lipofuscin pigment (asterisks) was also seen.

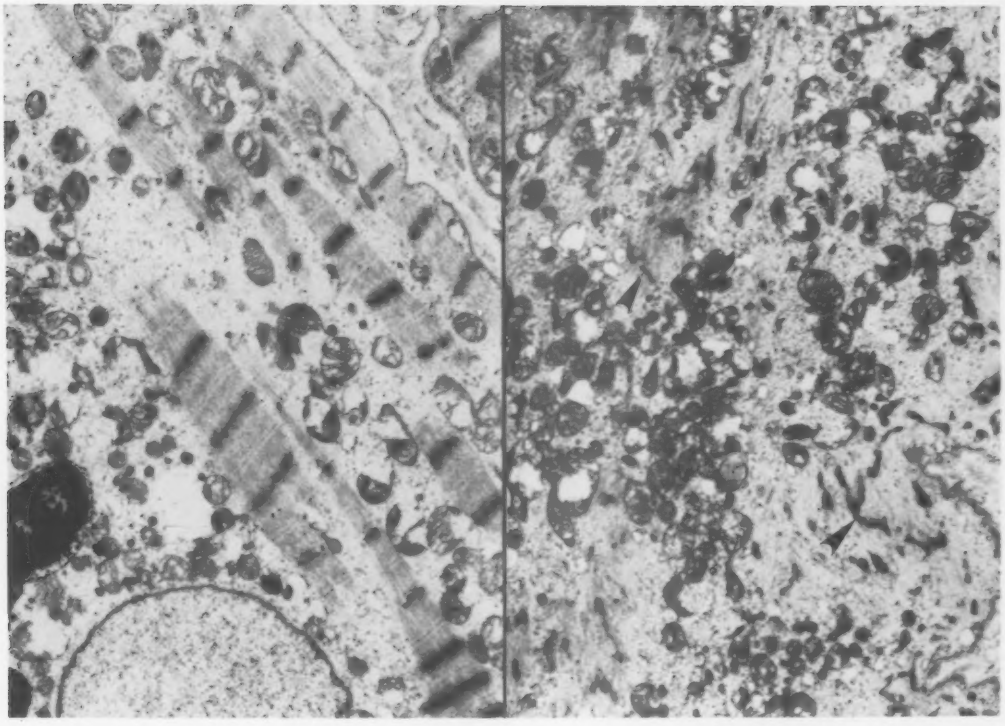

FIGURE 4.—Electron micrographs (both × 8750) from the same patient as fig 3. However, at the time of this biopsy she had received 220 mg/m^2 of mitoxantrone. Left side demonstrates more severe mitochondrial degeneration. Right side shows total loss of myofibrils. Some Z-band material remains (arrowheads).

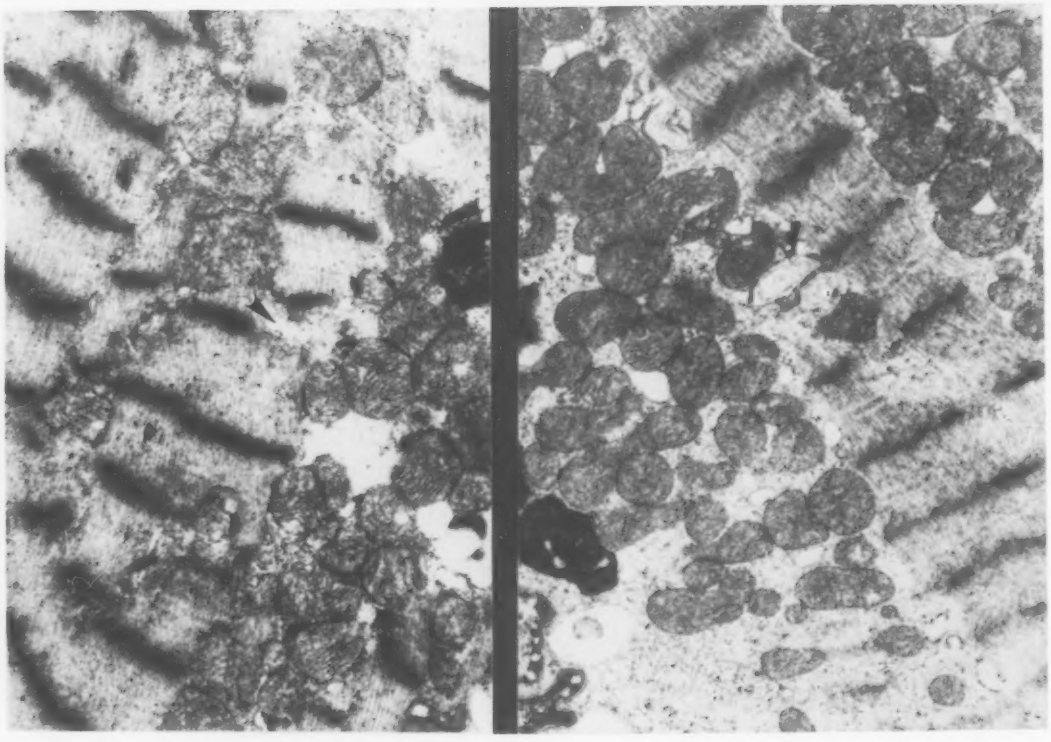

FIGURE 5.—Left panel shows myocardium (× 15,000) of a patient who had received 80 mg/m^2 of doxorubicin. There is some swelling of tubular structures (arrowhead). Right panel shows myocardium from the same patient after he then received 122 mg/m^2 of mitoxantrone. Myocardial alterations were no different in this second biopsy specimen.

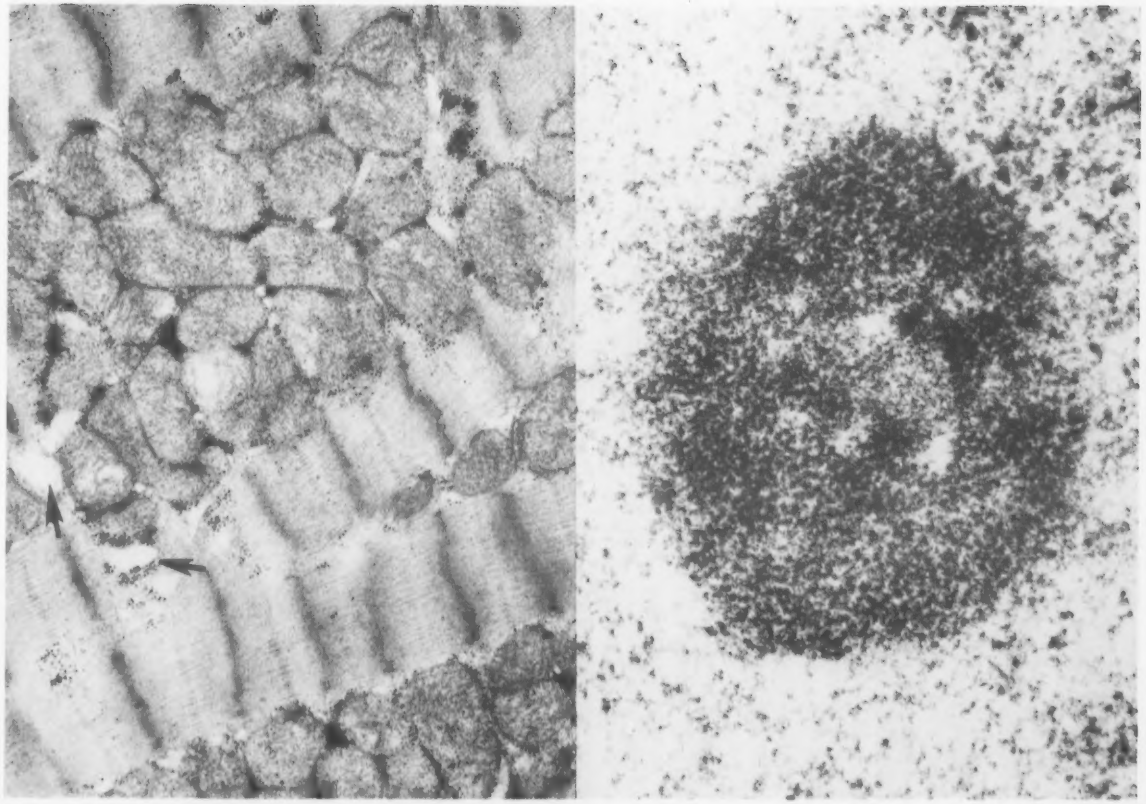

FIGURE 6.—Myocardium of a patient who had received 4675 rads to her chest for breast cancer and then 130 mg/m^2 of mitoxantrone. Left panel shows the myofibrillar area of the cell (× 22,000). Mild swelling of tubular structures (arrows) is seen. Mitochondria are normal. Right panel shows a contracted nucleolus (× 80,000) that is consistent with decreased nuclear activity. Such changes can be seen with radiation alone.

DISCUSSION

Clinically evident heart failure developed in one of these 18 patients and mild but definite impairment of cardiac function appeared during mitoxantrone therapy in the group as a whole. Deterioration of cardiac function did not appear to be more severe in the group that had received prior cardiotoxic agents, but the number of patients studied was small. The one patient who did develop heart failure had not received prior cardiotoxic chemotherapy. However, she had received a very large total cumulative dose (208 mg/m^2) of mitoxantrone.

The morphologic changes noted in the myocardial cells of mitoxantrone-treated patients were qualitatively similar to the changes noted after doxorubicin therapy. The nuclear changes including the chromatin clumping, nucleolar contraction, and degeneration were similar to doxorubicin-treated cells (10) and are consistent with the DNA-reactive nature of these drugs. The swelling of mitochondria and tubular structures in the mitoxantrone-treated patients was also similar to that seen in doxorubicin-treated myocardial cells. Mitoxantrone-induced alterations were not severe except for the one patient who had received > 200 mg/m^2. In addition to the changes noted above, her myocardium demonstrated myofibrillar lysis (fig 4).

The results of this study indicate that mitoxantrone is cardiotoxic. Although the cardiotoxicity is mild in total cumulative doses < 100 mg/m^2, congestive heart failure has been produced in one patient who had received no prior cardiotoxic chemotherapy but had received > 200 mg/m^2 of mitoxantrone. Therefore, close monitoring of cardiac function is indicated for all patients receiving mitoxantrone therapy. A direct comparison of mitoxantrone with doxorubicin is necessary to determine their relative toxic to therapeutic ratio.

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Negative-Ion Chemical-Ionization Mass Spectrometry of Aclarubicin Analogs and Characterization of Two Metabolites in Man[1,2]

Ronald G. Smith[3,*] Antonius A. Miller,[3,4] John A. Benvenuto,[3] Manuel Valdivieso,[3] and Ti Li Loo[3,5]

Aclarubicin and seven analogs have been characterized by negative-ion chemical-ionization mass spectrometry. The method is highly sensitive (requires 1–10 ng) because of the stable semiquinone radical anions that are produced by resonance electron capture of thermal electrons. Ions in the spectra correspond to the intact molecule (M), M-H_2O, aglycone, aglycone-O, and aglycone-$2H_2O$. In addition, ions corresponding to the sequential loss of carbohydrate groups are exhibited in the spectra of compounds with di- and tri-saccharides. Two aclarubicin analogs were isolated from a patient's plasma and were found to be bisanhydroaklavinone, F, and one or both of the epimeric reduction products of the L-cinerulose carbonyl, M_1 and N_1. [Cancer Treat Rep 67:351–354, 1983]

Aclarubicin is a triglycosidic anthracycline that has been isolated from fermentation broths of *Streptomyces galilaeus* (strain MA244-MI) (1). It has demonstrated antitumor activity in clinical trials in Europe and Japan and is now in clinical trials in the United States (2,3).

In addition to aclarubicin, 18 structurally related anthracyclines have been isolated from *S. galilaeus* (4,5). Several of these may also be metabolites of aclarubicin in mammalian species. Indeed, several of these compounds have been tentatively identified by high-performance liquid chromatography (HPLC) and thin-layer chromatography in urine, feces, blood, bile, and tissues of mice, rats, rabbits, and dogs (6). A mass spectrometric method using negative-ion chemical ionization has recently been developed for characterizing anthracycline antibiotics (7). The high sensitivity of this method and its ability to produce molecular and other characteristic ions should be useful for confirming aclarubicin metabolites isolated from biologic samples.

This paper describes the mass spectral characterization of seven aclarubicin analogs and its use to confirm the identity of two metabolites from the plasma of a patient who had received aclarubicin.

MATERIALS AND METHODS

Chemicals

Aclarubicin was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Aclarubicin analogs were provided by Sanraku-Ocean Co, Ltd, Fujisawa, Japan, and Chiba Cancer Center, Chiba, Japan. Solutions of each analog except bisanhydroaklavinone (F) were prepared by dissolving weighed amounts of the solid in known volumes of methanol and making serial dilutions. Solutions of F were similarly prepared in acetonitrile.

Instrumentation

The mass spectrometer used in this study was a Finnigan Model 3300F equipped with a chemical ionization source and positive-negative ion detection capability with a conversion dynode electron multiplier and interfaced with an Incos 2300 data system. Spectra were obtained in the negative-ion mode using methane gas (at 1.0 torr, uncorrected) to produce thermal electrons

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within the source. The samples were introduced into the ion source by drying small volumes (0.2–1.0 μl) of anthracycline solutions on the platinum tip of a desorption chemical-ionization probe (Vacumetrics, Ventura, CA) and inserting the probe directly inside the source. The probe tip was heated by a linear increase in current from 0 to 2 A at a rate of 2 A/minute.

HPLC analyses were performed with a Waters Associates liquid chromatograph equipped with a Model M6000 pump, a Model U6K injector, and a Scheoffel FS970 fluorescence detector with excitation wavelength at 236 nm and emission at 550 nm. Peak areas and retention times were determined by a Hewlett-Packard 3390A reporting integrator. Separations were achieved with a 5-μm Spherisorb ODS-2 (4.6 × 250 m) column (Custom LC, Houston, TX). The solvent system consisted of 3 ml of 0.2 M sodium dodecyl sulfate, 10 ml of glacial acetic acid, 90 ml of acetonitrile, and 100 ml of water and was diluted to 1000 ml with methanol.

Sample Preparation

Blood was drawn into tubes containing heparin, and the plasma was separated by centrifugation. Plasma (3 ml) was applied to 3-ml Clin Elut tubes (Analytichem International, Lawndale, CA) and eluted three times with 3 ml of ethyl acetate. The eluates were collected, combined, and evaporated under a stream of nitrogen. The residues were reconstituted with 300 μl of the mobile phase, and 50–100-μl samples were injected into the HPLC.

Metabolite Isolation

Eluate corresponding to chromatographic peaks was collected from the HPLC column. The fractions were rechromatographed using methanol as the mobile phase to check purity and to remove salts from the initial eluent. The samples were then dried under nitrogen and examined by mass spectrometry.

RESULTS AND DISCUSSION

Aclarubicin and the analogs shown in figure 1 possess the anthraquinone nucleus. In addition to exhibiting an intense fluorescence spectrum, which is exploited for HPLC detection, the anthraquinone nucleus provides intense negative ions under chemical ionization conditions. These are derived by resonance electron capture of thermal electrons generated by the reaction of primary electrons with the methane reagent gas within the ion source. The stability of the electron capture products is favored by the semiquinone radical anion (fig 2).

The main ions from the negative-ion chemical-ionization mass spectra of aclarubicin and seven analogs are listed in table 1. These spectra are characterized by semiquinone radical anions derived from the intact molecule (M), M-H_2O, aglycone, aglycone-2H_2O, and aglycone-O. In addition, aclarubicin and its analogs exhibit ions derived from sequential losses of the carbohydrate groups. This enables one to determine the molecular weights of all carbohydrate groups and their sequence. Sequencing of oligosaccharides by mass spectrometry has also been achieved by others (8,9).

The losses of water may be due to thermal or ionic degradations or both. Dehydration of the intact molecule is likely in the nonaromatic D ring in which the double bond is in conjugation with the anthraquinone system. Similarly, the elimination of the carbohydrate group and water from the anthracyclines forms the naphthacenequinone ring system. These processes are facilitated by carbohydrate groups, as evidenced by the intense ion at m/z 376 in the spectra of the analogs with carbohydrate groups. Conversely, dehydration is insignificant in the spectra of the aglycone, aklavinone (D_1), and 7-deoxyaklavinone (C_1). The aglycone-O ion is due to reductive cleavage of the carbohydrate moiety, a mass spectrometric process similar to those observed in chemical (10) and enzymatic (11,12) reactions of anthracyclines.

TABLE 1.—Negative ions in mass spectra of aclarubicin and analogs

Compound	m/z (% relative abundance)					
	M	M-H_2O	M-glycosides*	Aglycone	Aglycone-O	Aglycone-2H_2O
A	811 (5)	793 (1)	699 (0.5) 569 (2)	412 (5)	396 (9)	376 (100)
C_1	396 (100)	—	—	—	—	—
D_1	412 (100)	—	—	—	396 (2)	376 (1)
F	376 (100)	—	—	—	—	—
M_1	813 (6)	795 (2)	699 (1.4) 569 (0.9)	412 (8)	396 (15)	376 (100)
N_1	813 (5)	795 (0.8)	699 (1.3) 569 (3)	412 (10)	396 (16)	376 (100)
S_1	699 (6)	681 (1.0)	569 (1.4)	412 (6)	396 (15)	376 (100)
T_1	569 (23)	551 (3)	—	412 (4)	396 (21)	376 (100)

*Ions which still possess L-rhodosamine.

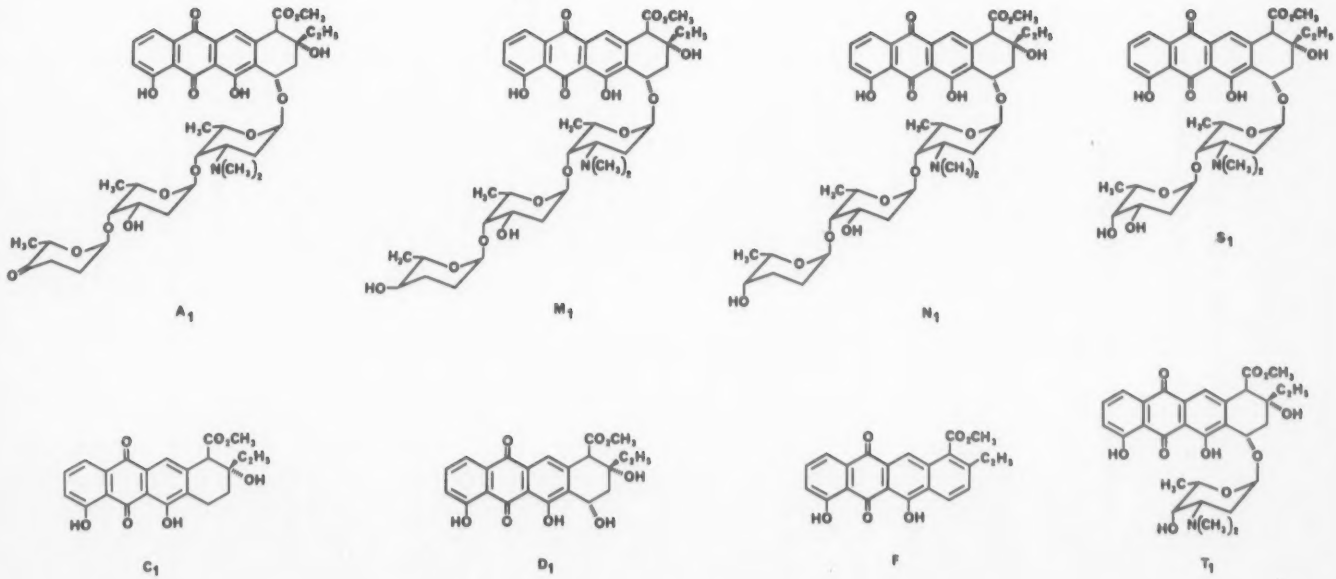

FIGURE 1.—Structures of aclarubicin (A_1) and the 7 analogs examined in the study.

FIGURE 2.—Resonance electron capture of thermal electrons.

The epimers derived from the reduction of the keto group of L-cinerulose, M_1 and N_1, give essentially identical spectra and are thus indistinguishable by this method.

Negative-ion chemical-ionization mass spectrometry is highly sensitive to anthracyclines (7). Like other members of this class of compounds, aclarubicin and its analogs provide useful spectra from 1–10-ng samples. Molecular ions are observed in these spectra despite their low relative abundance.

Two aclarubicin metabolites have been confirmed in the plasma of a patient treated with this agent. These were first observed by HPLC; retention times were consistent with those obtained for authentic samples of F and either M_1 or N_1. The metabolites were collected from HPLC fractions and desalted by rechromatographing with methanol as solvent. Examination of these samples by negative-ion chemical-ionization mass spectrometry gave spectra identical to those obtained from the authentic samples of F and M_1 or N_1. The partial hydrolysis products, S_1 and T_1, and M_1, have been reported as aclarubicin metabolites in a dog (11). This report was based on HPLC retention times. Thin-layer chromatography has been used to detect M_1, N_1, S_1, and T_1 as well as the aglycone metabolites C_1 and F in rabbit blood (12). In another report two fluorescent metabolites were observed in patient plasma (13). One of these cochromatographed with F while the other did not cochromatograph with any previously described aclarubicin analog.

Negative-ion chemical-ionization mass spectrometry is a valuable technique for the characterization of aclarubicin and its metabolites. It is highly sensitive and provides ions indicative of the molecular weight, the carbohydrate groups present, and the aglycone. This method should also be useful for characterizing new metabolites of aclarubicin.

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Differential Sensitivity of Pancreatic Adenocarcinoma Cell Lines to Chemotherapeutic Agents in Culture[1,2,3]

Barbara K. Chang[4,*]

Progress in chemotherapy for pancreatic adenocarcinoma has been hampered by the lack of appropriate tumor models; such models have only recently become available (1). Because of the need for improvement in chemotherapy for pancreatic adenocarcinoma, screening of a number of chemotherapeutic agents was undertaken against a panel of cell lines: two of human origin (PANC-1 and COLO-357) and two of hamster origin (WD PaCa and PD PaCa). Of the 14 drugs tested, aziridinylbenzoquinone (AZQ), menogarol (7-con-*O*-methylnogarol), and mitoxantrone emerge as worthy of further investigation against pancreatic adenocarcinoma.

MATERIALS AND METHODS

Cell Lines

PANC-1 and COLO-357 are of human origin. PANC-1 was obtained from the American Type Culture Collection and is an adherent cell line derived by Lieber et al (2) from a poorly differentiated ductal tumor. COLO-357 was obtained from G. E. Moore, University of Colorado (3), and is an adherent cell line from a well-differentiated ductal adenocarcinoma. WD PaCa and PD PaCa, both of hamster origin, were obtained from D. G. Scarpelli and M. S. Rao, Northwestern University (4,5). WD PaCa is a well-differentiated pancreatic ductal adenocarcinoma that grows as a solid tumor in vivo and was adapted to tissue culture prior to use in these experiments. PD PaCa is a poorly differentiated pancreatic ductal adenocarcinoma that grows as an ascitic model in vivo and readily grows as a single-cell suspension in vitro. All cell lines form colonies in semisolid media. Average doubling times in our laboratory under routine conditions of passage are as follows: PANC-1, 24–36 hours; COLO-357, 36–48 hours; WD PaCa, 36 hours; and PD PaCa, 18 hours. The adherent cell lines (PANC-1, COLO-357, and WD PaCa) are passaged and harvested by short exposure to 0.25% trypsin.

Drugs

The following agents were tested: dactinomycin, AZQ, 1,1-cyclobutanedicarboxylatodiammineplatinum (CBDCA), cisplatin, carboxyphthalato-1,2 diaminocyclohexaneplatinum (DACH), doxorubicin, 5-FU, menogarol, methotrexate, mitoxantrone, spirogermanium, streptozocin, vinblastine, and NSC-328005 (PALA derivative of cyclohexanediammineplatinum). The following agents were supplied by the Developmental Therapeutics Program, National Cancer Institute: AZQ, CBDCA, DACH, and NSC-328005 (Drug Synthesis and Chemistry Branch) and menogarol, mitoxantrone, and streptozocin (Natural Products Branch). The remaining agents were obtained commercially.

Dose-Survival Assays

For dose-survival determinations, cells were harvested in late log or early stationary phase growth. A total of 1–2 × 10^6 cells were incubated at 37°C in 5% CO_2 for 1 hour with drug concentrations of 0.01, 0.1, 1.0, 10, and 100 μg/ml. Cells were washed free of drug and resuspended in complete medium [RPMI-1640, with 15% fetal bovine serum, glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) (GIBCO, Grand Island, NY)]. In 35-mm culture dishes (LUX, Naperville, IL), colony formation (clonogenic) assays were carried out in quadruplicate using cell concentrations of 1–2 ×

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TABLE 1.—ID50s (in μg/ml) of chemotherapeutic agents tested against 4 pancreatic adenocarcinoma cell lines

Drug	PANC-1	COLO-357	WD PaCa	PD PaCa
Dactinomycin	0.16	0.24	1.1	0.35
AZQ	1.0	1.6	0.8	1.4
CBDCA	140	39	11.7	39
Cisplatin	13.0	2.0	2.8	0.15
DACH	10.5	42.0	30.5	4.9
Doxorubicin	0.025	37.0	4.0	0.42
5-FU	27	6.2	12.0	12.0
Menogarol	0.9	15.2	1.5	0.21
Methotrexate	> 100	> 100	> 100	> 100
Mitoxantrone	0.013	100	0.93	0.13
Spirogermanium	13.5	13.0	13.0	12.5
Streptozocin	> 100	> 100	> 100	> 100
Vinblastine	1.3	0.19	57	13.5
NSC-328005	24	61.0	3.5	61.0

10^3/ml in complete medium containing 0.9% methylcellulose; colonies were enumerated after 5–10 days.

Drug assay conditions (1 hour) were chosen to represent, as nearly as possible, pharmacokinetic conditions in vivo, where most drugs have short half-lives and are rapidly cleared from plasma (6). It was felt that longer periods or continuous exposure may overestimate drug responsiveness since such conditions cannot be realistically achieved in vivo. It is recognized that certain drugs (eg, methotrexate) may require longer incubations and further testing to determine their activity (7). However, for many experimental agents, pharmacologic data are not available, and a standardized 1-hour exposure has the merit of providing a well-defined cell culture dose, taking both concentration and time into account. The drug concentrations were chosen to provide a spectrum of exposures, based on (*a*) the consideration that most chemotherapeutic agents have molecular weights between 100 and 1000, so that the maximum dose tested, 100 μg/ml, corresponds to 0.1–1 mM, and (*b*) the recognition that to be active, substantial inhibition of colony formation should be seen within the concentration range of 0.1–10 μg/ml (0.1 μM–0.1 mM). The 0.01-μg/ml concentration is included mainly as a negative control (and also to screen for the possible response to very low drug concentrations), and the 100-μg/ml concentration is included mainly as a positive control.

Results are expressed as the surviving fraction, or T/C ($\bar{X}$ colonies treated/$\bar{X}$ colonies control) for each drug concentration. ID50s-1 hour (ie, the drug concentration, for 1-hour exposure, at which there is a 50% inhibition compared to controls) were determined from the log dose-survival curves.

RESULTS

The results of the dose-survival determinations for a number of standard and experimental agents against the two human cell lines and the two hamster cell lines are summarized in table 1 and, for selected agents, depicted graphically in figures 1–4. At present, well-accepted criteria for defining in vitro drug sensitivity are unavailable. Ideally, such criteria would take into account pharmacokinetic parameters achievable in vivo. Unfortunately, the relevant pharmacokinetic information is not available for most experimental agents. Use of a measure such as the ID50-1 hour at least provides a means for intercomparison of cell lines and drugs. From previous in vivo studies (1), we know that WD PaCa is resistant in vivo to dactinomycin, doxorubicin, 5-FU, methotrexate, and streptozocin, with PD PaCa being sensitive to doxorubicin and resistant to streptozocin. Unpublished observations suggest in vivo sensitivity of PD PaCa to cisplatin and dactinomycin and resistance of WD PaCa to cisplatin. The data from the present studies may be summarized as follows: (*a*) PD PaCa and PANC-1 are our most and second most chemosensitive cell lines, respectively; (*b*) WD PaCa and COLO-357 are our most resistant cell lines, with chemoresistance corresponding to degree of differentiation and slower cellular proliferation; and (*c*) the agents showing the most promise, based on their broader spectrum of activity against the four cell lines, are AZQ, menogarol, and mitoxantrone. Dactinomycin is discounted because of its known in vivo inactivity against WD PaCa (1) and human pancreatic cancer (8). The low ID50s achieved with dactinomycin may be seen as a reflection of its potency; a meaningful cell culture dose would correspond to 0.182 μg-hour/ml based on its concentration × time in man (9).

DISCUSSION

Given the heterogeneity of human cancer, the use of a panel of cell lines should offer distinct advantages in selecting agents that merit further study. The tumor models chosen for the present study appear to be ap-

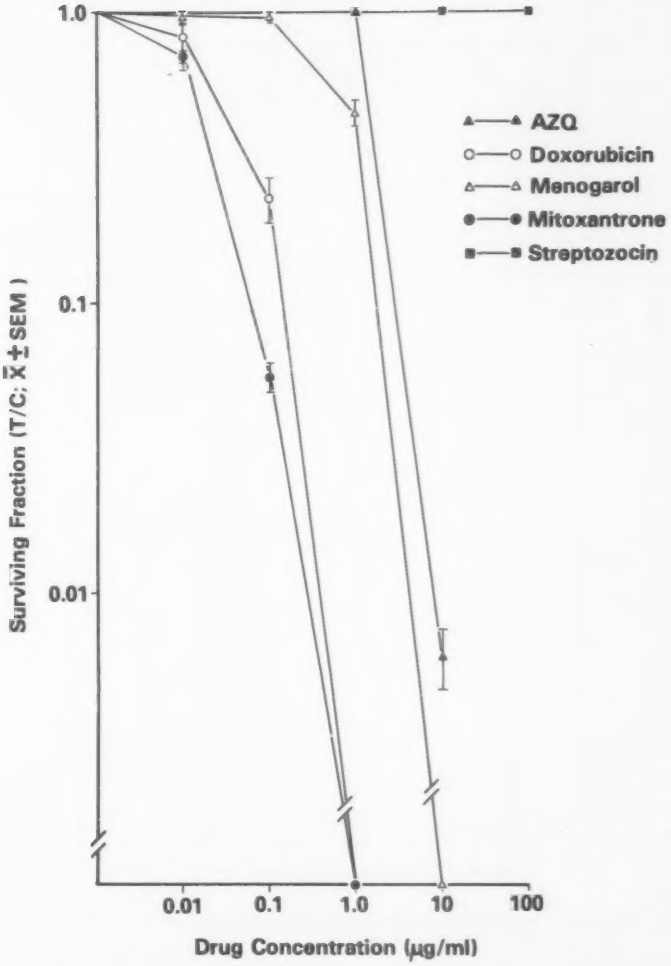

FIGURE 1.—Representative in vitro dose-survival curves of human PANC-1 cells assessed by the colony-formation assay.

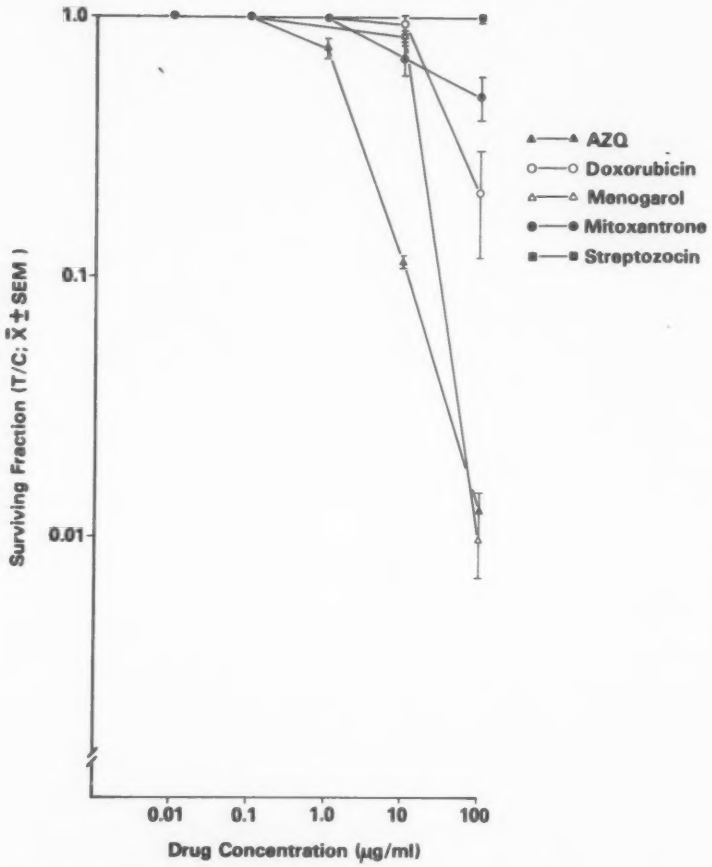

FIGURE 2.—Representative in vitro dose-survival curves of human COLO-357 cells assessed by the colony-formation assay.

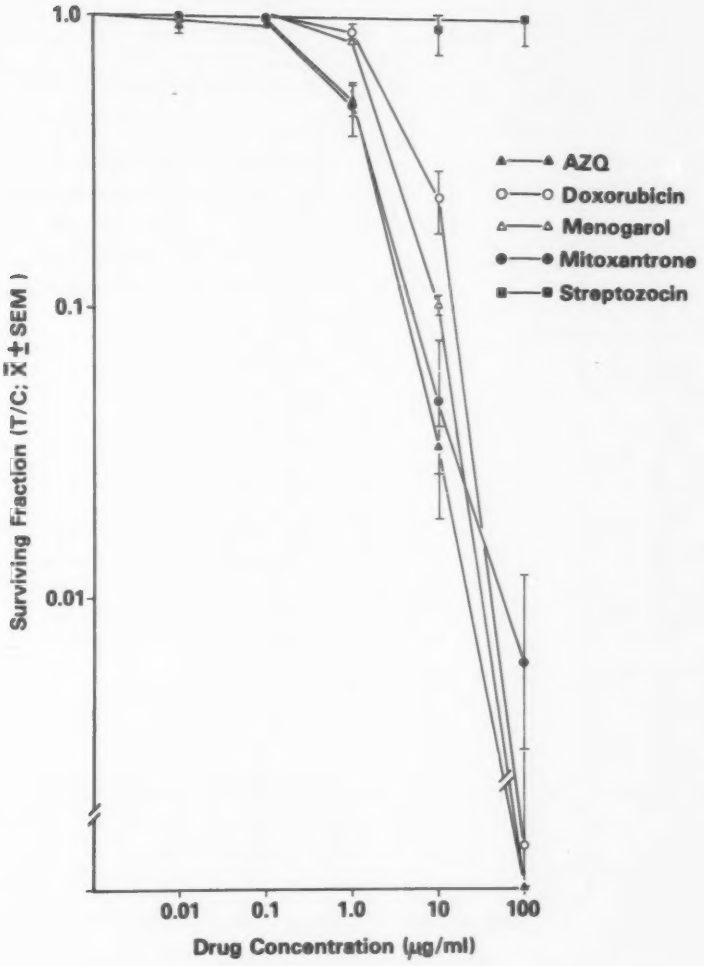

FIGURE 3.—Representative in vitro dose-survival curves of hamster WD PaCa cells assessed by the colony-formation assay.

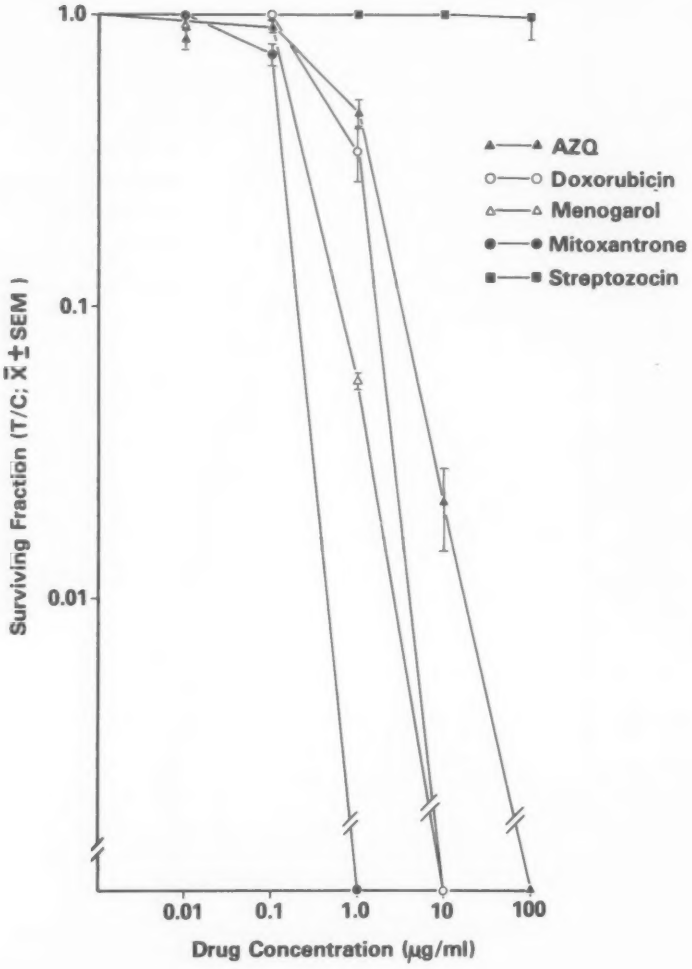

FIGURE 4.—Representative in vitro dose-survival curves of hamster PD PaCa cells assessed by the colony-formation assay.

propriate ones, not only in terms of their histology [since 75% of nonendocrine pancreatic cancers are classified "ductal" (10)] but also in terms of the relative refractoriness of three of the four cell lines to chemotherapeutic agents; this is very reminiscent of the human situation in which most agents are ineffective and objective responses are seen in only 13%–28% of patients even with the most effective single agents (11). There is also some evidence from clinical trials to suggest that the more poorly differentiated pancreatic adenocarcinomas are more sensitive to chemotherapeutic agents (12), as was the case with our cell lines.

While extrapolation from the in vitro to the in vivo situation is always difficult, we are able to make such comparisons with the two hamster models, as described previously, and with athymic (nude) mouse pancreatic cancer xenografts. Nevertheless, it would be presumptuous to suggest that agents identified as effective using these pancreatic tumor models will be active against human pancreatic cancer. However, we are encouraged by the broad spectrum of in vitro activity of AZQ, menogarol, and mitoxantrone. Demonstration of antitumor activity against highly resistant tumor models often presages a wide therapeutic spectrum, which has been suggested in early animal screens for AZQ (13), menogarol (14,15), and mitoxantrone (16). At the present time, we are pursuing in vivo studies with these three agents. Due to the slow pace, the expense, and the difficulties of evaluating the response of pancreatic cancer to antineoplastic agents encountered in phase II clinical trials (17), there is ample justification for utilizing an experimental approach to pancreatic cancer, which may aid in the identification of agents worthy of further investigation.

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Sensitive Enzyme Immunoassay for the Quantification of Bleomycin Using β-D-Galactosidase as a Label[1,2]

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A sensitive and specific enzyme immunoassay (EIA) for an anticancer drug, bleomycin (BLM), has been developed, which allows measurement of as little as 25 pg of the antibiotic/tube. An antibody to BLM was obtained by immunizing rabbits with an antigen prepared by conjugating BLM with mercaptosuccinylated bovine serum albumin via *N*-(γ-maleimidobutyryloxy)succinimide as a coupling agent. Enzyme labeling of BLM was performed using β-D-galactosidase (β-Gal; EC 3.2.1.23) via *N*-(*m*-maleimidobenzoyloxy)succinimide. Free and antibody-bound BLM-β-Gal conjugates were separated by precipitation of the antibody-bound fraction with a second antibody (anti-rabbit IgG). Displacement of BLM-β-Gal by unlabeled BLM when plotted as a logit-log function was linear over a concentration range of 10 pg–1 ng. The antibody distinguished alterations in the terminal structure of BLM, showing 277% cross-reaction with BLM B_2, 4.6% with A_2, 0.42% with A_2'-b, 0.13% with A_5, and 0.07% with the second-generation analog peplomycin. The EIA is free from interference by other anticancer drugs. Using this assay, drug levels were easily determined in tissues of rats following sc administration at a dose of 500 μg/kg. The sensitivity and specificity of the EIA should provide a useful tool for developing pharmacokinetic and toxicity studies of BLM. [Cancer Treat Rep 67:363–369, 1983]

The antibiotic bleomycin (BLM), isolated from the culture filtrate of *Streptomyces verticillus* (1), exhibits potent antitumor activity against a variety of neoplasms such as squamous cell carcinoma, lymphoma, and testicular carcinoma (2,3). Although it induces minimal bone marrow toxicity, administration of BLM is associated with significant pulmonary and mucocutaneous toxicity (4). Pharmacokinetic data will be useful for its optimal clinical usage. Thus, several methods such as the isotopically labeled BLM (5) and microbiologic assay (6,7) techniques have been used to study the clinical pharmacology of BLM. A radioimmunoassay has also contributed significantly to an understanding of the pharmacology (8–10).

Recent studies have shown that enzyme immunoassay (EIA) may have a comparable sensitivity to radioimmunoassay (11) and overcomes drawbacks inherent in the radiolabeling (12–14). However, an EIA system for BLM has not previously been developed.

This report describes the production in rabbits of an antibody specific to BLM using a novel procedure for preparing the immunogen, the labeling of BLM with β-D-galactosidase to act as a tracer, and its use in an EIA capable of detecting 25 pg of the drug. Optimal assay conditions were established and the method was used to measure BLM levels in tissues of experimental animals.

MATERIALS AND METHODS

Materials.—Clinically available BLM hydrochloride, BLM A_2, B_2, A_2'-b, A_5, and peplomycin were supplied by Dr. W. Tanaka of Nippon Kayaku Co, Tokyo, Japan. β-D-Galactosidase (β-Gal; EC 3.2.1.23) from *Escherichia coli* was purchased from the Boehringer Mannheim Co, W Germany. *N*-(γ-Maleimi-

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dobutyryloxy)succinimide (GMBS) and *N*-(*m*-maleimidobenzoyloxy)succinimide (MBS) were synthesized by our previous methods (12,15).

Preparation of the immunogen.—The preparative process was similar to our method of synthesizing immunogens for peplomycin (15) (fig 1). Clinically available BLM (10 mg; approximately 7.7 μmols) in 1.0 ml of 0.1 M phosphate buffer, pH 7.2, was mixed with GMBS (0.25 mg; 0.89 μmols) in 0.5 ml of tetrahydrofuran and incubated for 100 mins at 25°C with stirring. After removing tetrahydrofuran by passing nitrogen through the reaction mixture, the aqueous solution was washed three times with 3 ml of ether-dichloromethane mixture (2:1, by vol) to remove any remaining free GMBS. Acetylmercaptosuccinyl bovine serum albumin (AMS.BSA; 10 mg), prepared by the method of Klotz and Heiney (16), was incubated in 0.2 ml of 0.1 M hydroxylamine, pH 7.2, at 25°C for 30 mins to remove the protecting acetyl group. The resulting mercaptosuccinyl (MS) BSA (MS.BSA), estimated to contain 17 thiol groups per BSA molecule (17), was diluted with 3 ml of 0.1 M phosphate buffer, pH 7.0, added immediately to the GMBS-acylated BLM solution, and then incubated for 30 mins with vigorous stirring. Purification of the conjugate was achieved by column chromatography on Sephadex G-100. The conjugate was estimated spectrophotometrically to contain approximately 3 mols of BLM per mol of BSA, assuming the molar extinction coefficients of BLM to be 12,600 at 280 nm and 14,500 at 295 nm, and those of BSA to be 43,600 at 280 nm and 11,900 at 295 nm.

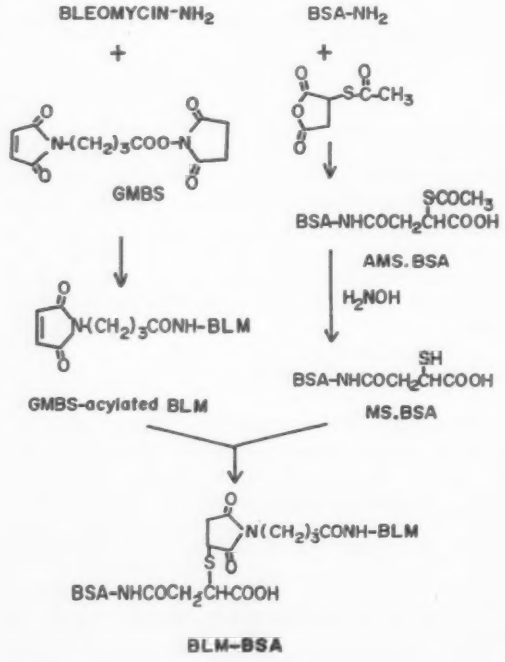

FIGURE 1.—Scheme for synthesizing immunogen for BLM.

Immunization.—The chromatographically purified BLM-BSA was used for production of antiserum in three white female rabbits by the procedure of Hurn and Landon (18). The rabbits received 1.0 mg of the conjugate, emulsified in complete Freund's adjuvant, by im and sc injection. This was followed three times by biweekly booster injections of 0.5 mg of the conjugate. The rabbits were bled from an ear vein 2 wks after each injection. Sera were separated by centrifugation, heated at 55°C for 30 mins, and stored at –30°C.

Enzyme labeling of BLM.—A solution of clinically available BLM HCl (0.2 mg) in 0.1 M phosphate buffer (pH 6.0, 1.0 ml) was incubated at 25°C for 30 mins with 50 μl of a tetrahydrofuran solution of MBS (2.0 μg). A solution of β-Gal (78 μg) in 0.1 M phosphate buffer (pH 6.0, 1.0 ml) was then added and the mixture was stirred for 30 mins. The reaction mixture was directly chromatographed on a Sepharose 6B column (2 × 38 cm) using 0.02 M phosphate buffer (pH 7.0) containing 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% BSA, and 0.1% NaN_3 (Buffer A) as an eluent. Fractions (3 ml) were collected, and the enzyme and immunoreactive enzyme activities of the conjugate were examined using 5 μl from each fraction by the method described below (fig 2). The immunoreactive enzyme activity was almost parallel with the pure enzyme activity. The immune specificity, defined as the immunoreactive enzyme activity in the presence of BLM (200 pg), showed that the conjugate was better differentiated from BLM in the later peak fractions than in the early peak fractions. Fractions 19–21 of the main peak were thus chosen from the EIA. The amount of immunoreactive enzyme activity of the conjugate formed was 25% of the enzyme activity. The conjugate was stable for > 1 year in Buffer A at 4°C without any loss of immunoreactive enzyme activity. A Km value of the substrate (7-β-D-galactopyranosyloxy-4-methylcoumarin) against the BLM-β-Gal conjugate was found to be 0.29 mM, which was not different from that of the pure enzyme.

EIA.—The buffer used for immune reaction consisted of 0.06 M sodium phosphate (pH 7.4) containing 0.01 M EDTA and 0.1% BSA (Buffer B); it was utilized for all dilution of antiserum and for samples or BLM standard solution. The assay was performed by the double-antibody method (19). Five microliters of BLM-β-Gal (130 μU), 50 μl of BLM or sample, as appropriate, and 50 μl of a 1/5000 solution of the antiserum were mixed and incubated for 8 hrs at 25°C. Fifty microliters of a 10% solution of goat anti-rabbit IgG and 50 μl of a 0.33% solution of normal rabbit serum were then added. At

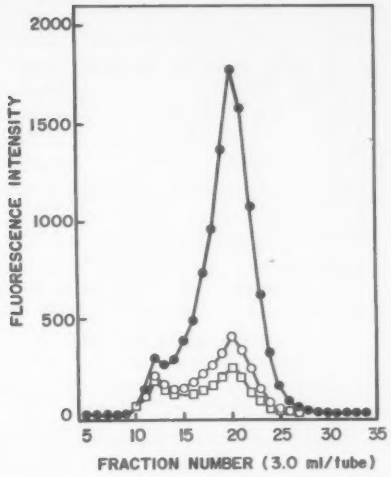

FIGURE 2.—Elution profiles of BLM-β-Gal from a Sepharose 6B column. ● = enzyme activity of the conjugate, measured by the use of 5 μl from each fraction at 30°C for 5 mins; ○ = immunoreactive enzyme activity, determined by the EIA described in the Materials and Methods section, except that 5 μl of the conjugate and a 1/1000 solution of anti-BLM serum were used in the absence of BLM; □ = immune specificity, determined by the same manner in the presence of 200 pg of BLM.

the end of the second incubation for 3 hrs at 25°C, 1.0 ml of Buffer A was added and thoroughly mixed. The mixture was then centrifuged at 2000 rpm for 20 mins at 4°C. The supernatant fraction was removed by decanting and the enzyme activity in the immune precipitate was measured.

Measurement of the enzyme activity.—Five microliters of diluted enzyme solution was incubated with 0.15 ml of 0.1 mM 7-β-D-galactopyranosyloxy-4-methylcoumarin in Buffer A at 30°C for 1 hr. The reaction was stopped by the addition of 2.5 ml of glycine-NaOH buffer (0.2 M; pH 10.3), and the 7-hydroxy-4-methylcoumarin liberated was measured spectrofluorometrically at wavelengths of 365 nm for excitation and 448 nm for emission. The amount of the conjugate was expressed in units of β-Gal activity, defining 1 unit of the enzyme activity as the amount that hydrolyzes 1 μmol of the substrate/min (20).

Determination of BLM levels in rat tissues.—Wistar rats weighing about 120 g were injected sc with BLM at a dose of 500 μg/kg and were killed by decapitation 2, 6, or 12 hrs after the injection. Tissues were removed and homogenized in vols of ice-cold 5% trichloroacetic acid using a Polytron Model PT 10 (Brinkman Instruments, Westbury, NY). The homogenates were centrifuged at 4°C for 15 mins at 2500 rpm, and the supernatants were collected, adjusted to pH 7.2 with 1.0 N NaOH, and used to quantify BLM by the EIA. Samples of tissues and body fluids obtained from control rats injected with saline were processed in the same manner as those obtained from rats injected with BLM, and they were included in the EIA as controls.

RESULTS

Preparation of the Immunogen for BLM and of BLM-β-Gal Conjugate

The conjugation method is based on the reaction of a maleimide group coupled to BLM and a sulfhydryl group of a protein (MS.BSA or β-Gal) by thiolation (*see* fig 1 for immunogen preparation). Considering the instability of the maleimide group of the coupling agent (GMBS or MBS), a large molar excess of BLM to the coupler was allowed to react in the first-step reaction so that the succinimidyl ester of the coupler might react rapidly and completely with an amino group of BLM. In the second step, the maleimide group of the coupler carrying BLM was directly conjugated with the thiol group of the protein (MS.BSA or β-Gal) under aqueous neutral conditions. Thereafter, the conjugate (BLM-BSA or BLM-β-Gal) was easily separated from the excess or nonreactive small molecular compounds by gel filtration techniques.

Antibody Response

An antibody against BLM was produced in each of three rabbits immunized with a BLM-BSA conjugate and was detected by the reaction of diluted antiserum with BLM-β-Gal conjugate. The serum collected 2 weeks after each injection was tested for its binding ability to BLM-β-Gal conjugate. BLM-β-Gal was incubated with solutions of the antiserum (or prebled serum) for 6 hrs, and this was followed by further incubation for 3 hrs with the second antibody (goat anti-rabbit IgG and normal rabbit serum). The uptake of BLM-β-Gal in the precipitate served as a quantitative measure of bound antibody. Typical binding curves are shown in figure 3 for serum from bleedings of one of the three rabbits. The antibody titer increased gradually with booster injections and reached a maximum 2 weeks after the final injection. No binding ability was seen with prebled serum.

Enzyme Immunoassay for Bleomycin

A dose-response standard curve obtained in Buffer B is shown in figure 4. The curve was essentially linear on a logit-log plot between 10 pg and 1 ng. The lowest detectable amounts of BLM which were significantly

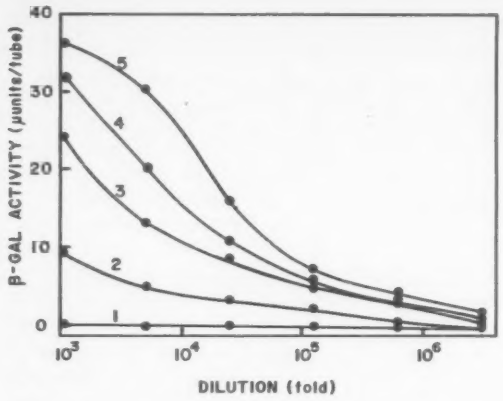

FIGURE 3.—Quantitative determination of anti-BLM antibody. Samples 2–5 are the antisera collected 2 wks after each injection. Sample 1 was prebled before immunization.

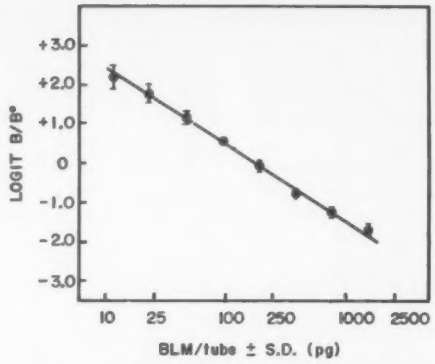

FIGURE 4.—Standard curve for the EIA of BLM. The response, Y, to the dose, X, can be defined as a logit function of Y so that Y = ln(Y/1-Y). Y is the logit of the ratio of antibody-bound enzyme activity to enzyme activity at 0 concentration of unlabeled BLM. Thus, the response logit B/B° to dose, log BLM concentration is plotted. Each point represents the mean ± SD for 6 replicates.

different from zero varied from 5 to 10 pg. For practical purposes, the lower and upper limits of sensitivity were arbitrarily set at 25 pg and 1 ng/assay tube, respectively. Intra-assay precision, defined as the ratio between the standard deviation of Y and the slope of the regression line between mean values of logit Y and the corresponding log X, was $\lambda = 0.031$ at 175 pg. In these experiments the specific binding of the tracer to anti-BLM antibody in the absence of nonlabeled BLM (B°) was 25.1% ± 1.0% (mean ± SD).

Detection of BLM in 10 μl of serum samples was also performed by adding known concentrations, and yielded almost the same standard curve as that yielded by Buffer B. Interassay quality control data in the presence or absence of serum are shown in table 1, revealing that this newly developed EIA is a reproducible technique. The interassay coefficient of variation, at seven different levels of BLM, ranged from 6.0% to 25% in the buffer systems and from 5.5% to 25.1% in the serum systems.

Antibody Specificity

The antibody specificity was determined by measuring the displacement of bound BLM-β-Gal by several BLM analogs such as BLM A_2, A_2'-b, A_5, B_2, and a second-generation BLM analog, peplomycin. As illustrated in figure 5, the anti-BLM serum showed 277% cross-reaction with BLM B_2, 4.6% with A_2, 0.42% with A_2'-b, 0.13% with A_5, and 0.07% with peplomycin when comparing the drug amount required to inhibit BLM-

TABLE 1.—Reproducibility of the dose-response curve assessed by interassay coefficient of variation in 10 different assays

	Added (pg/tube)	Estimated* (pg/tube)	Recovery (%)	Coefficient of variation (%)
BLM interassay in buffer B	25	28.0 ± 7.0	112	25.0
	50	54.0 ± 6.6	108	12.2
	100	106 ± 10.2	106	9.6
	200	206 ± 12.3	103	6.0
	400	390 ± 25.0	97.5	6.4
	800	758 ± 55.2	94.8	7.3
	1600	1680 ± 380	105	22.6
BLM interassay in the presence of serum (10 μl)	25	27.5 ± 6.9	110	25.1
	50	58.9 ± 7.8	118	13.2
	100	103 ± 10.0	103	9.7
	200	195 ± 10.8	97.5	5.5
	400	395 ± 31.1	98.8	7.9
	800	788 ± 66.3	98.5	8.4
	1600	1650 ± 402	103	24.4

*Mean ± SD.

β-Gal binding to the antibody by 50% with that of a clinically available BLM mixture. We also examined the effect of other anticancer drugs on the EIA for BLM. No cross-reaction was seen with the following drugs: doxorubicin, mitomycin, dactinomycin, 5-FU, methotrexate, cytarabine, and zinostatin (data not shown).

Comparison of the sensitivity of BLM detection between the EIA and a microbiologic assay using *Bacillus subtilis* EIYO was also made and showed that the lower limit of detection by the microbiologic assay was such that 1 μg/ml of BLM in a 0.08-ml volume could be measured. This is 2000 times less sensitive than the EIA. It was also found that there was a good correlation between results by the microbiologic and the EIA methods ($\gamma > 0.95$; $n = 20$) (data not shown). The biologic assay, however, was less accurate when BLM was present in serum, owing to the effect of serum proteins.

Experiments were also designed to measure the change in immunoreactivity of BLM following incubation in normal human serum or urine. In these fluids, known concentrations of BLM (25 pg–10 ng) were incubated for up to 24 hrs at 37°C, and the samples were evaluated at timed intervals. Five specimens were tested in triplicate experiments, and recoveries of BLM from serum and urine samples were 94.5% ± 5.5% (SE) and 95.8% ± 4.2%, respectively, of the added drug, by the EIA determinations.

Tissue Distribution of BLM

BLM levels in tissues of rats at 2, 6, or 12 hrs after sc injection of a limited amount (500 μg/kg) of the antibiotic were measured using the EIA for BLM (table 2). High levels of BLM in the kidney and an apparent peak at the 6-hr point suggest excretion of a large amount of antibiotic into the urine. Indeed, the excretion of BLM equivalents in the urine (based on the total dose administered) was about 36.9% in 12 hrs. No BLM was detected by the EIA in heart, spleen, or muscle at any time interval (data not shown). Of note is the fact that in liver, stomach, or lung the moderate amount of BLM seen in the first 2 hrs decreased rapidly by 6 hrs, while there was not much difference in kidney, intestine, brain, pancreas, or thymus between the concentrations at 2 and 6 hrs.

DISCUSSION

In view of the potential importance of BLM as a cancer chemotherapeutic agent, it is important to develop a specific, sensitive, and easy assay for the detection and quantification of BLM in clinical pharmacologic studies.

We recently developed an EIA for the anticancer drug peplomycin, a semisynthetic second-generation BLM analog, using the heterobifunctional cross-linking agents GMBS and MBS for preparing the immunogen and for the β-Gal-labeling of the drug, respectively (15). The assay was highly sensitive and specific to peplomycin, and it hardly detected its parent agent BLM. The success of this procedure prompted us to develop systematically an EIA specific for BLM.

To conjugate BLM to the protein (MS.BSA or B-Gal) rapidly and completely using the chemically rather unstable coupler (GMBS or MBS), we modified the procedure slightly, so that a large molar excess of BLM is

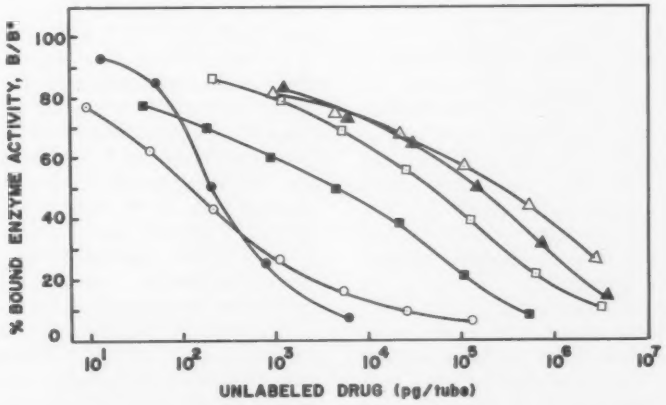

FIGURE 5.—Specificity of the BLM antiserum. The proportion of antibody bound to BLM-β-Gal is plotted as % bound: commercially available BLM mixture (●); BLM A_2 (■); BLM B_2 (○); BLM A_2'-b (□); BLM A_5 (▲); and peplomycin (Δ).

TABLE 2.—Tissue distribution of BLM in rats

Tissue*	Time (hrs)	BLM (ng/g tissue)	Tissue (g/rat)	BLM (ng/organ)
Kidney	2	54.0	1.320	71.3
	6	64.5	1.917	124.0
	12	21.3	1.474	31.3
Intestine	2	6.34	4.851	30.8
	6	7.92	5.733	45.4
Brain	2	8.12	1.644	13.3
	6	4.64	1.802	8.3
Thymus	2	19.1	0.470	8.9
	6	24.0	0.656	15.8
Pancreas	2	4.82	0.711	3.4
	6	6.71	0.960	6.45
Lung	2	23.7	0.793	18.7
	6	1.54	1.055	1.62
Stomach	2	21.5	0.855	18.5
	6	2.20	1.246	2.74
Liver	2	16.7	7.888	132.0
Tongue	2	7.12	0.368	2.6
Urine	2		10 μg† (12.4%)	
	6		33.6 μg† (32.4%)	
	12		30.0 μg† (36.9%)	
Blood	2		54.8 ng/ml	

*Each organ was examined for BLM levels at 2, 6, or 12 hrs after sc injection. However, only the levels in a detectable range by the EIA were given, omitting lower levels impossible to detect. BLM levels and tissue weights are the mean from the 2 rats.

†Total values.

allowed to react with the coupler in advance of the thiolation reaction with the protein.

An anti-BLM serum was obtained following immunization of rabbits with the BLM conjugate of MS.BSA using GMBS as a coupler (figs 1 and 3).

BLM-β-Gal conjugate was also prepared, with full retention of enzyme activity, by essentially the same two-step procedure as used in the preparation of the immunogen for BLM, using MBS structurally different from GMBS, to eliminate any antibody binding to the cross-linkage region of β-Gal (15). This β-Gal-labeling of BLM and its subsequent purification could be performed more easily, rapidly, and safely within 1 day compared with the cases of radioactively labeled BLM (^{125}I-BLM), in BLM radioimmunoassay (8,10). Further, the conjugate is stable for $>$ 1 year in Buffer A, while ^{125}I-BLM should be prepared every 2 months because of the short half-life of radioactivity.

An EIA for BLM can detect as little as 25 pg of BLM/tube and is reproducible as shown by an overall mean interassay coefficient of variation of 12.7% at seven different levels of BLM. This assay appears to be 2000 and ten times more sensitive than the corresponding microbiologic assay and BLM radioimmunoassay (8), respectively. The present anti-BLM serum was highly specific for BLM but cross-reacted with components of the BLM mixture, distinguishing alterations in the terminal structure of the molecule (fig 5). Of the BLM analogs tested, the cross-reactivity is the highest with BLM B_2 (277%) followed by A_2 (4.2%), which are the main components in the clinically available BLM mixture used for preparing the immunogen (9). Minor components such as BLM A_2'-b, A_5, or a semisynthetic analog peplomycin showed almost no cross-reactivity. Of note is the fact that the dose-response standard curve for the clinical mixture is significantly steeper than that for any of the analogs tested (fig 5), thus indicating that the EIA is more suitable for measuring the clinical mixture than each of the BLM analogs. No cross-reactivity was seen with other anticancer drugs as well, including dactinomycin, doxorubicin, mitomycin, 5-FU, methotrexate, cytarabine, and zinostatin, indicating that the EIA for BLM is appropriate for use in clinical evaluation of combination chemotherapy, since BLM is often used in combination with other chemotherapeutic agents.

The presence of serum in the EIA system does not seem to interfere with BLM determination, since standards prepared in either buffer or serum systems yielded essentially the same values. It was also shown that with this EIA the immunoreactivity of BLM is not affected by incubating the drug in serum or urine for 24 hrs at 37°C.

Distribution studies of BLM in animals have previously been reported using microbiologic assay techniques or ^{3}H-labeled BLM after much larger doses of the antibiotic than doses commonly used for the treatment of human cancers (6,7,21,22). In the present study, a limited amount of BLM (500 μg/kg), comparable to the therapeutic doses in man, was administered sc to rats, and the present EIA determined a minute amount of the drug in several tissues of the rats. The fact that a major portion of the administered antibiotic is excreted by the kidney appears to be in accord with the results previously reported (6,7,21,22). Moreover, > 2 hrs after injection, the disappearance of BLM from liver, stomach, or lung is more rapid than that from any of the other tissues tested, suggesting the possibility that BLM might be metabolized rapidly in these tissues and the metabolites must cross-react with the antibody to an insignificant degree. It has been shown using microbiologic assays that BLM is inactivated more readily by incubation with homogenates of mouse liver, spleen, or stomach than homogenates of any other organ (6,7,21). Further studies of the cross-reactions of anti-BLM antibody to its metabolites are now in progress and will be reported elsewhere.

The EIA described in this report should provide clinical investigators with a new means for quantifying minute amounts of BLM in pharmacologic studies, with the additional benefits of a stable labeled antigen, easily available equipment, and no hazard from the labeled compounds.

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High-Dose Cytarabine in Acute Leukemia: Toxicity and Pharmacokinetics[1]

Yves Pommier, Laurence Pochat, Jean-Pierre Marie, and Robert A. Zittoun[2,*]

High-dose cytarabine (HDARA-C) at doses ranging from 1000 to 3000 mg/m² administered as 30-min iv infusions was used in 12 patients with acute leukemia. HDARA-C toxicity was marked by nausea, vomiting, and somnolence; fever occurred in one patient. Myelosuppression was brief and reversible; the wbc count nadir occurred between Days 10 and 15 after treatment. In this study of a limited number of patients, no reliable conclusions could be drawn about antileukemic activity. However, *(a)* HDARA-C appeared to be a well-tolerated regimen in acute myeloblastic leukemia in complete remission; *(b)* a clear improvement was obtained in a patient with central nervous system leukemia; and *(c)* a sharp but transient decrease in peripheral blast cell counts was seen in two patients with acute myeloblastic leukemia. Cytarabine distribution was bi- or tri-compartmental; plasma final half-life was > 4 hrs in six patients. Pharmacokinetic parameters were not correlated with serum deoxycytidine deaminase activity. HDARA-C crosses the blood-brain barrier and may be useful in the prophylaxis against and treatment of central nervous system leukemia. [Cancer Treat Rep 67:371–373, 1983]

Cytarabine (ARA-C) is widely used in the treatment of acute leukemia (1) and is, like methotrexate, one of the antileukemic drugs which crosses the blood-brain barrier (2). However, resistance to conventional doses of ARA-C is frequent. This may be due to a failure of malignant cells to activate ARA-C to its active metabolite ARA-C triphosphate (ARA-CTP) (3) or to the inactivation of ARA-C to uracylarabinoside (ARA-U) by deoxycytidine deaminase (CdR-DA) (4).

High-dose ARA-C (HDARA-C) could increase the intracellular concentration of ARA-CTP and saturate CdR-DA. Moreover, previous studies have shown the clinical efficacy and the acceptable toxicity of HDARA-C (5,6).

These data prompted us to further investigate the clinical pharmacology of HDARA-C (1000–3000 mg/m²) in patients with poor-prognosis acute leukemia. The relationship between serum CdR-DA and ARA-C pharmacokinetic parameters was also studied.

MATERIALS AND METHODS

Twelve patients with leukemia were entered in this study. There were nine males and four females, with median age of 32 years (range, 16–52). Seven patients had acute myeloblastic leukemia (AML), four had poor-prognosis acute lymphoblastic leukemia (ALL), and one had central nervous system leukemia (CNS-L) without bone marrow involvement after the remission of a blastic crisis of chronic myelogenous leukemia. Among the seven patients with AML, five were in complete remission (CR) and HDARA-C was used as a consolidation therapy.

HDARA-C was given as 30-min iv infusions. Doses were 2500–3000 mg/m² except in the one patient with CNS-L and in two patients with long remissions of AML (Patients 2 and 3); they received 1000, 1500, and 1000 mg/m², respectively (table 1). Patients with AML and the patient with CNS-L were treated with four HDARA-C infusions at 12-hr intervals. Cycles of treatment were repeated every 4–12 weeks. Patients with ALL received only one infusion as part of a protocol of the European Organization for Research on Treatment of Cancer.

The ARA-C pharmacokinetic study was performed using a radioimmunoassay for ARA-C determination. The sensitivity of the method was 1 nM (0.25 ng/ml) and cross-reactivity with ARA-U was < 0.01% (7). Sampling tubes contained 50 μg of tetrahydrouridine and were kept in ice to block plasma CdR-DA. Venous blood

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TABLE 1.—HDARA-C pharmacokinetics in plasma

Patient No.	Disease	Dose (mg/m^2)	CdR-DA	C_0 (μM)	Volume of distribution (L/m^2)	C_{240} (μM)*	α-half-life (mins)	β-half-life (mins)	γ-half-life (mins)
1	AML-CR	2600	0.48	2400	4.3	0.16	12	40	—
2	AML-CR	1500	11.30	2000	3	0.40	3	12	240
3	AML-CR	1000	16	240	17	1.40	13	45	—
4	AML-CR	3000	8.7	2800	4.3	0.15	2	14	130
5	AML-CR	2700	11.3	14	1000	0.32	15	95	—
6	AML	2800	4.2	680	17	0.80	10	75	—
7	AML	2600	5.5	168	50	0.28	10	90	250
8	ALL	2500	4.5	1600	6.3	0.60	2	36	160
9	ALL	2500	4.1	280	33	0.60	6	26	—
10	ALL	2400	3.9	200	50	0.06	9	36	—
11	ALL	2500	4.4	1600	6.3	0.28	10	98	250
12	CNS-L	1000	3.8	8	500	0.06	2	18	340

*C_{240} = ARA-C concentration 240 mins after the end of infusion.

was obtained just prior to ARA-C infusion and 1, 5, 15, 30, 40, 50, 60, 90, 120, 180, and 240 mins after HDARA-C. Cerebrospinal fluid (CSF) was obtained by lumbar puncture in three patients 60 mins after HDARA-C.

Serum CdR-DA activity was assayed by the conversion rate of ARA-C to ARA-U in 30 mins at 37°C (8) and was determined in all patients just before treatment.

RESULTS

Toxicity

Repetitive nausea was seen in 75% of the patients. Vomiting was usually controlled with antiemetics (metoclopramide) and stopped at the end of the ARA-C infusion. It was persistent or severe in only three patients. A sedative effect was common but could not be related to HDARA-C since antiemetics, which are known for their sedative effect, were administered concurrently. Headache occurred in four patients but three of these had a lumbar puncture 1 hr after HDARA-C. Patient 2 had fever (38°C–39°C) between 2 and 4 hrs after the third and the fourth HDARA-C infusions of each cycle. Wbc counts were always normal at these times and no source could be determined for fever. Myelosuppression occurred in all the patients. Neutropenia was achieved between Days 10 and 20 (nadir range, 1000–1800 wbcs/μl) after each HDARA-C cycle in the five patients with AML in CR. Thrombocytopenia occurred earlier (nadir about Days 10–12, with 70,000 platelets/μl). Wbc counts returned to normal within 10 days of the nadirs. There was no cumulative toxicity. Neither renal nor hepatic abnormalities were observed. However, cytomegalovirus hepatitis occurred in two patients with AML in CR several weeks after the beginning of HDARA-C cycles.

Pharmacokinetics

Plasma concentration at the end of HDARA-C infusion (C_0) varied from one patient to another (average, 1000 μM; range, 8–2800). These variations of C_0 were not correlated with plasma ARA-C clearance or serum CdR-DA (table 1). Plasma concentration 240 mins after HDARA-C was less variable (average, 0.43 μM; range, 0.06–1.40). Plasma ARA-C disappearance curves were bi- or tri-exponential leading to a bi- or a tri-compartmental open model (table 1). The first, second, and third half-lives (mean ± SD) were α-half-life = 7.8 ± 4.7; β-half-life = 48 ± 32; and γ-half-life = 228 ± 75 mins, respectively. Rapid plasma clearance of ARA-C was not related to a high CdR-DA activity (table 1).

ARA-C concentration in the CSF 60 mins after HDARA-C infusion was relatively high (3.12, 0.55, and 0.47 μM in Patients 8, 9, and 10, respectively) (table 2). The ratios of ARA-C concentrations in CSF and in plasma at this same time were similar in the three patients (0.13) (table 2).

DISCUSSION

The present study confirms that HDARA-C toxicity is acceptable and marked by nausea, vomiting, somnolence, fever, and myelosuppression (5). Moreover, the use of HDARA-C in patients with AML in CR showed

TABLE 2.—ARA-C concentration in the CSF 60 mins after the end of infusion (C_{60} CSF) and comparison with plasma concentration at the same time (C_{60} plasma)

Patient No.	C_{60} CSF (μM)	C_{60} plasma (μM)	$\frac{C_{60}\text{ CSF}}{C_{60}\text{ plasma}}$
8	3.12	24	0.13
9	0.55	4	0.14
10	0.47	4	0.12

that HDARA-C myelosuppression was brief and reversible and never led to septic or hemorrhagic complications in these patients. It appears therefore that outpatients can be treated with HDARA-C and that repetition of such cycles was tolerated without cumulative toxicity. However, we observed two cases of cytomegalovirus hepatitis several weeks after the beginning of HDARA-C cycles. Large doses of ARA-C within a short period of time have been reported to increase the risk for development of DNA virus infections owing to depression of host defenses by the drug (10) and might be responsible for these two cases.

Most of the HDARA-C pharmacokinetic data are in agreement with those of studies done with conventional doses of ARA-C: (*a*) the distribution was bicompartmental (2,10–12) or tricompartmental (13); (*b*) C_0 was about 100 times higher than after conventional doses, but the doses were also 100 times higher; and (*c*) ARA-C pharmacokinetic parameters were not correlated with serum CdR-DA activity but CdR-DA is known to be mainly intracellular (4). On the other hand, we found a particularly long terminal half-life of ARA-C (γ-half-life = 228 mins). It is difficult to explain this last finding by interferences from ARA-U in the radioimmunoassay since cross-reactivity was $< 0.01\%$ (7) and the ARA-C/ARA-U ratio has been shown previously (2) to be about 1% after HDARA-C. The association of this low ARA-C clearance and high C_0 could allow an increased exposure of malignant cells to the drug and thereby improve ARA-C therapeutic activity. Similarly, ARA-C concentrations in the CSF were high after HDARA-C, reaching the levels achieved in plasma after conventional doses (2,10–12). This therapeutic regimen could therefore be useful in the prophylaxis against and treatment of central nervous system leukemia.

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Cyclophosphamide, Vincristine, Lomustine, Cisplatin, and Doxorubicin in the Treatment of Non-Small Cell Lung Cancer[1]

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Fifty-four patients (47 of whom were evaluable) with non-small cell lung cancer were treated with a five-drug regimen consisting of cyclophosphamide, vincristine, lomustine, cisplatin, and doxorubicin. Six complete and 16 partial responses were achieved, for an overall response rate of 47% (22 of 47 patients). Response by cell type was as follows: epidermoid carcinoma, 41% (seven of 17 patients); adenocarcinoma, 42% (eight of 19); and large cell carcinoma, 64% (seven of 11). Response in patients with limited disease was 48% (11 of 23 patients) and in patients with extensive disease, 46% (11 of 24). Previously untreated patients had response rates of 53%, versus 22% in those with prior therapy.
[Cancer Treat Rep 67:375–376, 1983]

In 1979, Takita et al (1) reported a 65.7% response rate in non-small cell lung cancer using the drug regimen of cyclophosphamide, vincristine, lomustine (CCNU), cisplatin, and doxorubicin. Since that report, however, Whitehead et al (2) noted a response rate of only 17% with this combination, and Takita's group abandoned it because of toxicity (3). In contrast, we have found this regimen to be reasonably well-tolerated and effective. This report summarizes our experience.

MATERIALS AND METHODS

Between December 1979 and November 1981, 54 patients with non-small cell lung cancer were treated with the five-drug protocol at the Denver Veterans Administration Hospital. Twenty-six patients had stage III M_0 disease (18 had compromised lung function prohibiting aggressive radiotherapy), while 28 had stage III M_1 disease. All patients were male. Ages ranged from 27 to 83 years, with a median of 67 years. All patients had creatinine clearances > 50 ml/minute, wbc counts > 5000/mm^3, and platelet counts > 100,000/mm^3.

Chemotherapy was given at 4–6-week intervals and during a 12–18-hour hospital stay. Drug doses in patients < 65 years old, with a Karnofsky performance score (KPS) > 60%, were 300 mg/m^2 of cyclophosphamide iv, 1.4 mg/m^2 of vincristine iv, 50 mg/m^2 of CCNU orally, 50 mg/m^2 of cisplatin iv, and 50 mg/m^2 of doxorubicin iv. In patients > 65 years old, or with a KPS < 60%, the doses were reduced to 300 mg/m^2 of cyclophosphamide iv, 1 mg/m^2 of vincristine iv, 40 mg/m^2 of CCNU orally, 40 mg/m^2 of cisplatin iv, and 40 mg/m^2 of doxorubicin iv. Cisplatin was given by 6-hour infusion and was followed by hydration/diuresis for 8–12 hours.

Response was assessed after two courses of chemotherapy by an independent radiologist (Paul E. Siebert) and before any irradiation of a monitor lesion. In patients with measurable disease, complete response was defined as total regression; partial response was defined as a > 50% reduction in the product of the two greatest perpendicular tumor diameters lasting 2+ months. When evaluable but not readily measurable disease existed, response criteria similar to those of Eagan et al (4) were used: complete response = total regression; and partial response = definite tumor reduction lasting 2+ months without disease progression elsewhere. Tumor reductions of lesser degree, treatment deaths, and refusal to continue therapy were all considered nonresponses.

Responding patients received a total of five to six courses of chemotherapy. Those patients with limited

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disease, or only brain and lung involvement, then received thoracic irradiation (3000 rads/ten fractions); those with other sites of involvement received nothing more. In patients presenting with brain metastases, this treatment plan was preceded by whole-brain irradiation, but not irradiation of the primary tumor. Following completion of chemotherapy/radiotherapy, no further treatment was given until disease progression.

All patients were evaluated for survival; only those who remained evaluable for two or more courses of chemotherapy were assessed for response, however. Patients lost to follow-up were considered dead of disease at the time such follow-up was done.

RESULTS

Seven patients were evaluated for survival but not response: three had malignant pleural effusions as the only evaluable disease; two were lost to follow-up after two courses; one died of bowel obstruction after one course; and one had resection after two courses. Response rates for the remaining 47 patients are shown in table 1. There were six complete and 16 partial responses, for an overall response rate of 47%. The response rate was similar using standard criteria (16 of 35 patients) or Eagan's criteria (six of 12), and was also about the same for patients with limited and extensive disease. However, patients without prior treatment (53% response rate) fared much better than did those with prior therapy (22% response rate).

Three patients (6%) refused more than one course of chemotherapy and two (4%) died of bone marrow suppression. Another eight patients had wbc count nadirs (at 12–14 days) that were $< 1500/mm^3$; however, none became septic and all tolerated further treatment once a 20% reduction in the doses of cyclophosphamide, CCNU, and doxorubicin was made. The remaining patients all had wbc count nadirs $> 1500/mm^3$ and tolerated all courses of chemotherapy well.

Nine patients (eight with limited disease, one with brain and lung disease) received thoracic irradiation following completion of chemotherapy. Three responders with limited disease refused irradiation.

Life-table analysis of survival in all 54 patients shows 12- and 18-month rates of 38% (ten of 26 patients) and 14% (three of 22), respectively, for limited disease and 32% (nine of 28) and 14% (four of 28), for extensive disease. Responders have 12- and 18-month survival rates of 68% (15 of 22 patients) and 23% (five of 22), whereas these rates are 16% (four of 25) and 8% (two of 24), for nonresponders.

TABLE 1.—Response by patient characteristics

	No. of patients	
Treated	54	
Evaluable	47	
Responding	22	(47%)
Epidermoid carcinoma	7/17	(1 CR, 6 PR)*
Adenocarcinoma	8/19	(2 CR, 6 PR)
Large cell carcinoma	7/11	(3 CR, 4 PR)
Limited disease	11/23	(4 CR, 7 PR)
Extensive disease	11/24	(2 CR, 9 PR)
KPS > 60%	18/33	(54%)
KPS < 60%	4/14	(28%)
Prior treatment†	2/9	(22%)
No prior treatment	20/38	(53%)

*CR = complete response; PR = partial response.
†Prior chemotherapy, 1 patient; prior radiotherapy, 8.

DISCUSSION

Although Whitehead et al (2) reported a response rate of only 17% using this combination, 55% (ten of 18) of the patients in that study had had prior treatment, as compared to 20% (nine of 44) in our study and only 3% (one of 35) in that of Takita et al (1). Our response rate in previously untreated patients was 53% versus 22% in those with prior therapy. Thus, this particular protocol would appear to be most effective when patients have not failed earlier treatments. It also seems that our program was better tolerated than that of Takita. Although two treatment deaths were observed, both occurred prior to the time that dose reductions for elderly and/or poor-performance patients were instituted. Since that time, all patients have tolerated their chemotherapy reasonably well.

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CAP (Cyclophosphamide, Doxorubicin, and Cisplatin) Regimen in Adrenal Cortical Carcinoma[1]

H. van Slooten and A. T. van Oosterom[2,*]

Eleven patients with advanced progressive metastatic adrenal cortical carcinoma, for whom no further standard therapy was available, were entered into a phase II study of cyclophosphamide, doxorubicin, and cisplatin (CAP) combination chemotherapy. Two partial responses and six disease stabilizations were observed. Patients with partial responses survived 18+ and 23 months; the median survival of patients with disease stabilization was 10+ months. The total group had a median survival of 10+ months. These results, together with the modest toxicity, suggest that further study of the combination is warranted.
[Cancer Treat Rep 67:377–379, 1983]

Adrenal cortical carcinoma (ACC) is a rare malignant tumor with an incidence of two per million population per year (1,2); it accounts for only 0.2% of all deaths from cancer (3). The presentation of patients with these tumors is widely variable. Sixty percent of such patients present with symptoms of excess hormone production; this, however, does not influence the natural history of the disease. Histologic grading also does not correlate with the behavior of this disease (4–6). The prognosis of localized tumors is good (7), but the median survival after diagnosis in advanced disease is reported to be only 2.9 months (7,8).

In patients presenting with resectable tumors, surgery is the treatment of choice. The role of radiation therapy in the management of ACC is not well-defined (5,9).

Therapy with mitotane has had wide application, but other cytotoxic drugs have been less completely studied. The results of treatment with mitotane are not uniform. Objective tumor regression has been reported in large series to be 34% (5) and 61% (10). These studies, however, used response criteria which varied widely from the now standard World Health Organization (WHO) criteria. The objective response rate using WHO criteria in our series of 34 patients treated with mitotane was found to be 23%, with a relatively low response rate in patients with a large tumor load. Results of other antineoplastic compounds are mentioned only occasionally in broad phase I–II studies or in case reports. Responses have been reported in individual patients treated with carmustine (BCNU) (11), cisplatin (12), doxorubicin (13), and with combinations of cyclophosphamide-doxorubicin (13) and 5-FU-methotrexate (14). In view of the scarcity of data and the successful application of the combination of cyclophosphamide-doxorubicin-cisplatin (CAP) in bladder and ovarian cancer (15,16), we decided to test this combination in advanced progressive ACC.

MATERIALS AND METHODS

Eleven patients, nine women and two men, with histologically proven progressive metastatic ACC, were entered into the study. Their age ranged from 18 to 56 years (median, 46). In four patients symptoms of hormonal activity were apparent (three with Cushing's syndrome, one with a virilizing syndrome).

The primary tumor had been resected in seven patients and had recurred in four. Three patients had developed metastases as well: one in lung, one in liver, and one in lung and bone. Three patients developed metastases only: all three in lung, two also in liver, and one in bone. In four patients excision of the primary tumor was impossible; all had liver metastases, one with lung and one with bone metastases. In one patient in whom radiotherapy was applied to the tumor bed, distant indication lesions were used for the evaluation of response to chemotherapy. Two patients had been

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treated with mitotane prior to entry into this study but demonstrated disease progression. The Karnofsky performance scores ranged from 50 to 90 (median, 70). Bone marrow, cardiac, and renal functions were adequate in all patients at the start of the treatment. All patients were informed about the objectives of the study and gave consent.

CAP treatment consisted of the iv administration of cyclophosphamide (600 mg/m^2), doxorubicin (40 mg/m^2), and cisplatin (50 mg/m^2), with adequate prehydration and posthydration. The treatment was repeated on Day 22 if blood cell counts and renal function allowed. WHO criteria of response were used. Treatment was discontinued when disease progression was observed. Duration of response and stable disease was measured from the start of the treatment until disease progression.

RESULTS

No complete responses were observed among the 11 patients entered into the study. There were two partial responses, one in a patient with an only partially resected tumor and one in a patient with lung and liver metastases and an unresectable primary; these lasted 6–9 months. Two patients had a short disease stabilization of < 3 months, with disease stabilized in lung metastases (one patient) and in lung metastases and local recurrence (one) before renewed progressive disease became apparent after four and five courses. Four patients had stabilization for 4–10 months, with stabilization in local disease and liver metastases (three patients) and in liver and lung metastases (one).

Three patients had only one course and died within 2 months after the start of treatment. The deaths were due to rapidly progressive growth of local disease and lung and liver metastases in one patient and local recurrence with liver and bone metastases in another. Septic shock due to drug myelotoxicity occurred in one patient, who had a local recurrence and lung metastases. The median survival for the whole group was 10+ months (range, 2–25+). The two patients who had partial response survived 18+ and 23+ months after the start of the CAP therapy. The four patients who showed stabilization for more than four courses survived 5, 8, 17, and 25+ months, despite progressive disease at study entry. None of these patients received additional chemotherapy. No correlation between response and clinical signs of hormonal excess can be made; 17-ketosteroids and 17-hydroxysteroids in 24-hour urine samples showed inconsistent changes during chemotherapy. The CAP regimen produced no unexpected toxicity. One patient developed fatal septicemia during granulocytopenia. Nonfatal granulocytopenia occurred in two patients. Mild nausea and vomiting and alopecia occurred in all patients, as did cumulative mild nephrotoxicity with a 5-ml average drop in creatinine clearance per course per patient (median, eight courses). Two patients developed cardiomyopathy after 12 and 15 courses of doxorubicin and subsequently received cyclophosphamide and cisplatin only. No ototoxicity was observed.

DISCUSSION

The patients entered into this study had progressive advanced ACC with bulky disease, which made them unsuitable candidates for the use of mitotane. Our previous experience with mitotane in this situation showed no responses among 20 patients treated, with a median survival of 4.5 months after the start of medical treatment. Our result differed only slightly from the 2.9-month survival reported by McFarlane (7) as the natural history of untreated advanced cases.

Among the 11 evaluable patients in our CAP study we observed two partial responses resulting in an objective response rate of 18%, with 90% confidence limits of 0%–37%. Of note, however, is the potential gain in survival with this regimen. These preliminary data appear promising, and further study with the CAP regimen appears to be warranted. A multicenter study may be needed for sufficient accrual.

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Improved Control of Chemotherapy-Induced Emesis by the Addition of Dexamethasone to Metoclopramide in Patients Resistant to Metoclopramide[1,2]

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One hundred and two patients with advanced solid tumors receiving chemotherapy were treated with metoclopramide (MCP; 100 mg) to prevent emesis. Twenty-six of 102 patients who had more than five episodes of vomiting were considered MCP-resistant and were randomized to receive MCP (100 mg) plus placebo versus MCP plus dexamethasone (DM; 48 mg) iv in a double-blind, crossover trial. The median number of vomiting episodes was 18 after MCP plus placebo and five after MCP plus DM ($P < 0.001$). The median duration of vomiting episodes was 40 hours after MCP plus placebo and 10 hours after MCP plus DM. Immediate toxicity of the antiemetics was not enhanced by the addition of DM to MCP. [Cancer Treat Rep 67:381–383, 1983]

Metoclopramide (MCP), a procaine derivative, has been shown to be similar or better than prochlorperazine in the prevention of chemotherapy (CT)-induced emesis (1–2). However, MCP has not been found to be effective in every study (3). Rich et al (4), Baker et al (5), and Lee (6) have suggested the benefit of glucocorticoids.

The purpose of this prospective, randomized double-blind trial was to compare MCP plus placebo versus MCP plus dexamethasone (DM) in patients with advanced solid tumors receiving CT who developed severe emesis resistant to MCP.

MATERIALS AND METHODS

Between May and September 1981, 102 patients were admitted to our Department to receive different regimens of CT. The mean age was 49 years; there were 46 male and 56 female patients. No patient had received previous CT. The location of primary tumor in the 102 patients is shown in table 1.

TABLE 1.—Primary tumor location in patients receiving MCP

Tumor	No. of patients (%)
Lung carcinoma	13 (12.7)
Breast carcinoma	36 (35.2)
Colon carcinoma	11 (10.7)
Testicular carcinoma	8 (7.8)
Soft tissue sarcoma	8 (7.8)
Ovarian carcinoma	7 (6.8)
Head and neck carcinoma	6 (5.8)
Stomach carcinoma	5 (4.9)
Lymphoma	3 (2.9)
Prostate glandular carcinoma	3 (2.9)
Choriocarcinoma	1 (0.9)
Uterine cervical carcinoma	1 (0.9)
Total	102 (100)

All patients received MCP at a dose of 100 mg iv (40 mg was given 30 minutes before CT and 60 mg was administered in three doses of 20 mg each, every 6 hours). After completing the CT cycle, every patient answered a questionnaire about the number and duration of vomiting episodes and the intensity and duration of nausea. To evaluate the intensity of nausea, a score of 0–4 was used, with regard to patients' symptoms and their ability to receive solid or liquid diets. The scoring and quantitation of vomiting was done by the investigators.

Patients who had five or more episodes of vomiting after the first (including MCP) were considered resistant to MCP and were randomized to receive MCP (100 mg) plus placebo versus MCP plus DM (48 mg) iv (24 mg given 20 minutes before CT and 24 mg administered 6 hours later). During the next cycle a crossover was made. Only the first two crossover cycles were evalu-

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TABLE 2.—Rate of response to MCP according to cytostatic treatment*

Drug*	No. of patients (%)		
	Total	MCP-susceptible	MCP-resistant
Doxorubicin	26	24 (92.3)	2 (7.6)
Cyclophosphamide	11	9 (81.8)	2 (18.1)
Doxorubicin + cyclophosphamide	17	9 (52.9)	8 (47)
Cisplatin	13	6 (46.1)	7 (53.8)
Doxorubicin + cisplatin	8	4 (50)	4 (50)
CMF regimen	14	12 (85.7)	2 (14.2)
Other	13	12 (92.3)	1 (7.6)
	—	—	—
Total	102	76 (74.5)	26 (25.5)

*Doxorubicin, 60 mg/m^2; cyclophosphamide, 600 mg/m^2; cisplatin, 100 mg/m^2; and CMF regimen—cyclophosphamide (600 mg/m^2) + methotrexate (30 mg/m^2) + 5-FU (600 mg/m^2).

ated. Statistical analysis was performed by the method of Hills and Armitage (7) for crossover trials, with a chi-square test for discrete variables.

RESULTS

Of the 102 patients, 76 had less than five episodes of vomiting after MCP, and 26 had more than five episodes of vomiting and were considered resistant. Response to MCP was better in patients receiving doxorubicin or the "CMF" regimen (cyclophosphamide, methotrexate, and 5-FU) than in patients receiving cisplatin alone or in combination (table 2).

MCP side effects were found in 16 of 102 patients (15.6%): 15 patients had mild sedation and one patient had extrapyramidal movements.

The 26 patients resistant to MCP were randomized to receive MCP plus placebo versus MCP plus DM. Four of these patients were not evaluable: one patient who had received cisplatin plus doxorubicin died before the completion of the trial, one who had received doxorubicin changed the dose of CT, and two who had received cyclophosphamide and cisplatin each changed the CT regimen because of progressive disease. Using paired analysis, no difference was found in nausea or vomiting between the MCP plus placebo group and the baseline results with MCP alone (table 3).

All of the 22 patients showed a better response to MCP plus DM than to MCP plus placebo (table 3). Nine of 13 patients (69%) who received CT regimens without cisplatin had less than five episodes of vomiting after MCP plus DM (five patients had no emesis); six of nine (66%) who received regimens containing cisplatin had less than five episodes of vomiting after MCP plus DM (two patients had no emesis); and 13 of 15 (86%) who had less than five episodes of vomiting after MCP plus DM continued responding to MCP plus DM during two more CT cycles.

The final results were not influenced by the sequence in which the patients received placebo or DM, according to the method of Hills and Armitage (7) for crossover trials.

DISCUSSION

Our findings with MCP suggest that it can be safe and useful in the prevention of CT-induced emesis in patients receiving doxorubicin, cyclophosphamide, or

TABLE 3.—Results after the completion of the crossover trial with MCP + placebo vs MCP + DM in 22 patients*

	MCP (baseline)	MCP + placebo	MCP + DM
Median No. of vomiting episodes	19	18	5
Median duration of vomiting episodes (hrs)	43	40	10
Median intensity of vomiting episodes (score)	3.4	3.5	1.5
Median duration of nausea (hrs)	57	52	16

*P values: nonsignificant for MCP (baseline) vs MCP + placebo; < 0.001 for MCP + placebo vs MCP + DM.

the CMF regimen. When the cytostatic drug used is cisplatin alone or in combination, the rate of response to this dose of MCP is poor. Higher doses have been shown to be more effective, mainly in patients receiving cisplatin (8). However, side effects like diarrhea, sedation, or extrapyramidal movements appear at a higher rate at these dose levels.

Our results with DM suggest that it can effectively reduce emesis from CT treatment in patients initially resistant to MCP. The association of MCP and DM did not increase the immediate toxicity of MCP when used as a single drug. DM has recently been found to ameliorate the side effects of MCP that have been seen with higher doses (8). We have not been able to reproduce these findings, mainly because of the small number of patients who received DM, and the lower incidence of side effects we found at this dose level of MCP.

Because of studies with MCP in high doses by Gralla et al (9), we are currently using a higher dose of MCP and a smaller dose of DM. Further follow-up is required to evaluate long-term toxicity to this combination. Smaller doses of DM might be useful for patients who show a very good response. Delayed emesis might be prevented by a third dose of DM. MCP might be continued orally in ambulatory patients, after receiving a second dose of DM. The oral dosage form would allow the present regimen to avoid hospitalization with CT regimens that are given on an outpatient basis.

Although the development of newer and better antiemetic drugs continues to be a priority, the combination of MCP and DM can provide an acceptable degree of relief to many patients in whom severe emesis persists despite more common treatments.

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Effect of Peplomycin Plus Carbazilquinone and Mitomycin on Non-Small Cell Carcinoma of the Lung[1,2]

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The effects of two chemotherapeutic regimens, peplomycin (PLM) plus carbazilquinone and PLM plus mitomycin, on non-small cell carcinoma of the lung were evaluated by a randomized controlled trial. Seven partial responses were observed in 53 evaluable patients, for an overall response rate of 13.2%. There was no difference in response rate or in median survival time between PLM plus carbazilquinone (11.5% and 32 weeks) and PLM plus mitomycin (14.8% and 25 weeks). Seven patients developed interstitial pneumonitis, and four died of pulmonary fibrosis. It was concluded that the chemotherapeutic regimens that include PLM are only marginally effective against non-small cell carcinoma of the lung, with relatively frequent pulmonary fibrosis. [Cancer Treat Rep 67:385–387, 1983]

A large number of combination chemotherapeutic regimens have been introduced for the treatment of non-small cell carcinoma of the lung (1–5). Initial enthusiastic reports of high response rates are frequently followed by reports of much lesser response rates in larger multi-institutional trials. Although it is conceivable that one or more of the currently utilized combination chemotherapeutic regimens is in fact beneficial to both the quality and duration of survival (4), it remains to be demonstrated that any combination chemotherapy is superior to no treatment. Until such a beneficial effect from combination chemotherapy can be confirmed, it appears to be particularly useful to test new anticancer agents or new combinations of active agents in patients with previously untreated non-small cell carcinoma, to best assess anticancer activity of these new agents and combinations. Peplomycin (PLM), a derivative of bleomycin, has been shown to exhibit less pulmonary toxicity (6). In a phase I study of PLM conducted from August 1976 to February 1977, the clinical maximal tolerable dose of PLM was determined to be 15 mg/m^2 weekly, and the total tolerable dose was determined to be 120 mg/m^2 (7). In a phase II study of PLM against non-small cell carcinoma of the lung, partial response was observed in three of 25 patients with squamous cell carcinoma (11%) (7). There were not enough data to evaluate the effect of PLM on adenocarcinoma and large cell carcinoma. In this study, patients with non-small cell carcinoma of the lung were randomly assigned to chemotherapeutic regimens consisting of PLM plus carbazilquinone (CQ), an alkylating agent derived from ethylene-imino-quinone, or PLM plus mitomycin (MMC).

MATERIALS AND METHODS

Fifty-seven patients who were admitted to the respiratory division of the National Cancer Center Hospital were entered in this study between April 1980 and March 1982. All patients had either histologic or cytologic proof of non-small cell carcinoma of the lung. None of the patients had had prior chemotherapy or radiotherapy. Patients were excluded from the study if their life expectancy was < 4 weeks and if their Eastern Cooperative Oncology Group (ECOG) performance score was 4. Patients whose Pao_2 level was < 70 mm Hg were also excluded from the study. Of the 57 patients with non-small cell carcinoma of the lung, four were ineval-

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uable for response because of pleural effusion. The principal characteristics of the 53 evaluable patients are listed in table 1. Patients with stage III M_0 and III M_1 were evenly distributed in both groups, and the distribution of performance scores was the same in the two groups. Patients were randomly assigned to receive one of two regimens. All three drugs were given by bolus iv injection weekly. In one course of treatment, PLM (15 mg/m²) was administered weekly four times, in both arms of the study. CQ (4–6 mg/m²) or MMC (8 mg/m²) was given weekly three times, 3 days after PLM administration.

Response to treatment was defined as a decrease in the diameter of a tumor objectively measurable on chest roentgenogram or by palpation. In this study, a complete response (CR) was defined as the total disappearance of the tumor for at least 4 weeks. A partial response (PR) was defined as a ≥ 50% decrease in the product of the longest perpendicular diameters of measurable lesions, lasting > 4 weeks, without the appearance of new lesions or growth of other measurable lesions. Minor response was defined as a 25%–50% reduction in tumor size without the appearance of new lesions or growth of other measurable lesions lasting > 4 weeks. Stable disease was defined as less than minor response without evidence of disease progression. Progressive disease was defined as any increase in measurable lesions or the appearance of new lesions. Patients with PR received one more course of the same regimen and other patients were given different chemotherapeutic agents. Survival times were calculated from Day 1 of treatment until death. Comparisons of survival were made by the two-tailed generalized Wilcoxon-Gehan test.

TABLE 1.—Characteristics of patients and response to treatment*

Characteristic	PLM + CQ†	PLM + MMC†
Total	26	27
Sex		
Male	18	21
Female	8	6
Age in yrs		
Median	63.6	61.4
Range	40–80	38–76
ECOG performance score		
0	7	5
1	11	15
2	5	5
3	3	2
Stage		
III M_0	9	8
III M_1	17	19
Histology		
Squamous cell carcinoma	6	7
Adenocarcinoma	17	17
Large cell carcinoma	3	3
Response		
PR	3 (11.5%)‡	4 (14.8%)§
Minor	3¶	2**
No change + progressive disease	20	21

*Unless specified otherwise, values = No. of patients.

†PLM (15 mg/m²) was administered weekly 4 times. CQ (4–6 mg/m²) or MMC (8 mg/m²) was administered weekly 3 times, 3 days after PLM administration.

‡Adenocarcinoma, 3 cases.

§Adenocarcinoma, 2 cases; large cell carcinoma, 2 cases.

¶Squamous cell carcinoma, 2 cases; adenocarcinoma, 1 case.

**Squamous cell carcinoma, 2 cases.

RESULTS

Seven PRs were observed during treatment of the 53 evaluable patients; the overall response rate was 13.2%. Minor response was observed in five patients, and there was no CR in this study. There were two and five PRs among 17 and 34 evaluable patients with stage III M_0 and III M_1 disease, respectively. Performance scores were 0 or 1 for seven responders. Among the 26 patients treated with PLM + CQ, there were three partial responders (11.5%) with adenocarcinoma and three minor responders with squamous cell carcinoma (two) and adenocarcinoma (one). Among the 27 patients treated with PLM + MMC, there were four partial responders (14.8%) with adenocarcinoma (two) and large cell carcinoma (two), and two minor responders with squamous cell carcinoma (table 1). The median survival times for 26 patients treated with PLM + CQ and for 27 patients treated with PLM + MMC were 33 and 25 weeks from the beginning of treatment, respectively.

There are two and eight survivors at the time of this report in the PLM + CQ and PLM + MMC groups, respectively, and the survival times for six survivors treated with PLM + MMC have not yet reached 33 weeks. The median survival time for 39 patients with PRs and minor responses plus those with stabilized disease was 33.5 weeks; median survival time for patients with progressive disease was 14 weeks ($P < 0.02$). The hematologic toxic effects were tolerable in all the patients of both groups. Anemia and leukopenia were observed in > 50% of the patients treated with either PLM + CQ or PLM + MMC. There were no differences in the incidence and degree of hematologic toxicity between the two groups. Seven patients developed interstitial pneumonitis and four died of pulmonary fibrosis. Five of seven patients had received PLM + CQ. Pa_{O_2} began to decrease in the sixth week in five patients and in the second week in two. All four of the patients who died of pulmonary fibrosis were > 65 years old. In addition to myelosuppression and pulmonary toxic effects, acute toxic effects such as nausea, vomiting, and fever occurred in about two-thirds of the patients. Stomatitis and dermatitis occurred in one-fourth of the patients.

DISCUSSION

Although advances have been made in the treatment of small cell anaplastic carcinoma of the lung, non-small cell carcinoma remains more refractory to therapy (1). In Japan, CQ and MMC as single chemotherapeutic agents have been reported to produce respective response rates of 21% (15 of 71 patients) and 18.9% (ten of 53) in patients with non-small cell carcinoma of the lung (8). MMC has proved to be modestly active against non-small cell carcinoma of the lung and has been incorporated into combination chemotherapeutic regimens (5,8). Homma and Niitani (7) reported that PLM (15 mg/m^2 weekly, four times) produced a response rate of 18% (two of 11 patients) against squamous cell carcinoma of the lung, and combination chemotherapy with PLM + CQ achieved a response rate of 34.8% (eight of 23) against squamous cell carcinoma and 28.5% (two of seven) against adenocarcinoma of the lung. In the study reported here, however, the rate of response to PLM + CQ was 11.5%, and no responders were observed among six patients with squamous cell carcinoma. The rate of response to PLM + MMC (14.8%) was no better than that to MMC alone. In this arm also, there were no responders among seven patients with squamous cell carcinoma. These results suggested that the addition of PLM did not improve the effect of either anticancer agent. Seven of the 53 patients developed interstitial pneumonitis, and four died of pulmonary fibrosis. In other words, these regimens did not induce a very favorable response, and they were relatively frequently associated with interstitial pneumonitis. The median survival time for patients with PR, minor response, and no change was longer than that for those with progressive disease. However, the survival time for patients with PR was no better than for patients with minor response and no change. These results cast doubt on the contribution of these chemotherapeutic regimens to the prolongation of the survival of patients.

In the experience of Gralla et al (4) at Memorial Sloan-Kettering Cancer Center, vindesine plus cisplatin is an active combination with a response rate of 43% and median survival times in the range of 1 year, and the activity of this regimen has been confirmed at several institutions (3). It is clear that vindesine is active against non-small cell carcinoma of the lung (9). We also found vindesine to be active against adenocarcinoma of the lung in Japan. We are now testing a combination of vindesine and MMC against non-small cell carcinoma, especially against adenocarcinoma and large cell carcinoma.

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Phase II Trial of Mitoxantrone in Refractory Acute Leukemia[1,2]

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Mitoxantrone was administered to 41 adults with refractory acute leukemia. The initial dose employed was 4 mg/m²/day × 5 days. Eventually a starting dose of 10 mg/m²/day × 5 days was used, with some patients receiving a subsequent course at 12 mg/m²/day × 5 days. Three patients had a complete response, one a partial response, and one hematologic improvement. Although antileukemia effect was more frequent at the higher doses, so was death following treatment. [Cancer Treat Rep 67:389–390, 1983]

Mitoxantrone (DHAD) is one of a number of anthraquinones synthesized to improve the antitumor activity and reduce the cardiotoxicity of the anthracyclines, which they resemble structurally. Because of activity in murine leukemias, including some that are resistant to doxorubicin (1–3), phase II trials of DHAD in the treatment of refractory acute leukemia were begun. The initial dose used was that recommended from a phase I trial conducted at this institution (4).

PATIENTS AND METHODS

Forty-one adults (median age, 32 years; range, 18–72) were entered in the study. Criteria for entry were: (*a*) bone marrow leukemic infiltrate (% blasts + promyelocytes × % cellularity × 100) > 50%, or (*b*) circulating blast cell count > 10,000/μl, or (*c*) extramedullary leukemia. Types of leukemia were: acute myelocytic (AML; 27 patients), acute lymphocytic (ALL; eight), lymphoma-leukemia (LL; four), and chronic myelocytic in blastic transformation (CML-B; two). Twelve patients (six with AML, one with ALL, three with LL, and two with CML-B) had never been in remission; the remainder had relapsed. Patients had received a median number of three prior induction regimens (range, one to five). Thirty-seven patients had been given anthracyclines, usually doxorubicin (median cumulative doxorubicin dose, 250 mg/m²; range, 100–690).

The dose of DHAD, administered iv over 1 hour, was the same each day for 5 days. The initial dose was 4 mg/m². Bone marrow aspiration and biopsy specimens were obtained before treatment and 14–17 days after the start of treatment. Patients who showed insufficient cytoreduction received a second course. If dose-limiting toxicity had not occurred, the dose for the second course was 5, and at a later date, 6 mg/m². The latter was used as the next starting dose, with escalation to 8 mg/m² in the second course. Starting doses of 8 and then 10 mg/m² were eventually employed, with subsequent doses being 10 and later 12 mg/m². Patients demonstrating significant marrow hypoplasia were not given increased doses in later courses.

Standard definitions of complete response (CR) and partial response (PR) were employed (5). Monitoring for cardiac toxicity was clinical only.

RESULTS

Three patients achieved CR (7%; 95% confidence range, 0%–16%). These included: (*a*) a 61-year-old man with AML who had failed initial induction therapy with two courses of doxorubicin, cytarabine, vincristine, and prednisone (OAP) plus doxorubicin immediately prior to DHAD; (*b*) a 63-year-old man with AML who had attained initial CR with rubidazone plus OAP, had relapsed during cytarabine maintenance, and had failed salvage therapy with high-dose cytarabine; and (*c*) a 29-year-old man with ALL who had achieved initial CR with doxorubicin plus OAP, had relapsed during OAP therapy, and had failed salvage therapy with doxorub-

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icin plus OAP, 6-thioguanine, and L-asparaginase. None of the three patients experienced significant toxic effects. CR durations were 10 months (unmaintained), 1 month, and 1 month (both maintained with DHAD), respectively. A PR and hematologic improvement (normal neutrophil count and bone marrow with 85,000 platelets/μl), each of 1 month's duration, occurred in two patients with LL. An additional 18 patients developed marrow aplasia; 12 of them died with aplasia and six had leukemic regrowth. Insignificant marrow cytoreduction was seen in 16 patients. Two patients died before antileukemia effect could be evaluated. Results were unaffected by morphologic diagnosis.

CR did not occur in ten patients[4] receiving low doses of DHAD (< 6 mg/m²). There were two CRs among 23 patients (9%; 95% confidence range, 1%–27%) given intermediate (6 or 8 mg/m²) compared to one CR among 22 patients (5%; 95% confidence range, 0%–23%) receiving high doses (> 8 mg/m²). Marrow aplasia occurred in one additional patient at low doses, in eight at intermediate doses, and in 13 at high doses. There were two induction deaths at low doses, compared to eight and 16 at the intermediate and high doses, respectively. Considering only the dose administered in the first course, results were the same. All deaths during induction were a result of infection and/or hemorrhage.

Median time from initiation of therapy to recovery of normal blood cell counts was 32 days (range, 22–54) in patients with CR, PR, or hematologic improvement. One-half of the patients who developed marrow aplasia without CR, PR, or hematologic improvement had hypocellularity lasting > 3 weeks from start of treatment (longest, 44 days), while the other one-half died or had leukemic regrowth prior to this time. A relation between dose and duration of aplasia could not be established. Mucositis was observed in 54% of 35 patients evaluable for extramedullary toxic effects, abnormal liver function tests were seen in 31%, and diarrhea occurred in 11%. Severe toxic effects were seen in 9%, 21%, and 35% of the patients as doses increased from low to intermediate to high. Six of seven cases of severe mucositis occurred at high doses; three of five instances of severe liver toxicity occurred at intermediate doses. Cardiac toxicity was not encountered. The median total dose of DHAD administered was 60 mg/m² (range, 20–150).

DISCUSSION

Our results with DHAD are approximately the same as those reported by Van Echo et al (6).

Comparison of these results with those for other single agents in the treatment of refractory adult acute leukemia at our institution reveals that the frequency of CR with DHAD approximates that with 5-azacitidine (7). The CR rates with both amsacrine (AMSA) (8) and rubidazone (9) were higher: 24% with the former and 33% with the latter. However, only six of the 41 patients given DHAD were receiving their initial salvage regimen, compared with 38 of 104 given amsacrine (8). Keating et al (10) found that the CR rate for phase II agents in AML is 20% when used as first salvage, 10% for second salvage, and 0% for third salvage. Thus, comparison of effectiveness is difficult.

Although antileukemia effect, as judged by occurrence of marrow aplasia, was more frequent at high doses, so was the incidence of death during induction. The latter relationship was not due to prolongation of marrow aplasia at high doses. Since 35% of patients given high doses had severe extramedullary toxic effects, usually mucositis, these patients possibly also had gut lesions predisposing to systemic infection.

DHAD has activity in adult acute leukemia. We recommend a dose of 6–8 mg/m²/day × 5 days.

[4]A patient was analyzed separately for each different dose received.

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Phase II Trial of Amsacrine (AMSA) in Urinary Bladder Cancer[1,2]

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Amsacrine (AMSA), a new acridine derivative, has demonstrated antitumor activity against a variety of tumors (1–3). Phase I studies indicated that 90–120 mg/m^2 iv every 3–4 weeks produced predictable and tolerable myelosuppression and occasional mild nonhematologic toxicity (1,3). Therefore, a disease-oriented phase II trial of AMSA in patients with objectively measurable metastatic bladder cancer was conducted and forms the basis of this report.

MATERIALS AND METHODS

Twenty-two patients (16 males and six females) with histologically confirmed bladder cancer received AMSA. Treatment eligibility criteria included bidimensionally measurable metastatic disease, wbc count $\geq$ 4000/mm^3, platelet count $\geq$ 100,000/mm^3, Karnofsky performance status (KPS) $\geq$ 50%, serum bilirubin $<$ 2.0 mg/dl, no chemotherapy in the preceding 3 weeks, and informed consent. All patients had a complete history and physical examination, an automated blood cell and platelet count, a 12-channel biochemical screening profile, and a chest roentgenogram. Radionuclide liver scans were performed in patients with hepatomegaly or abnormal biochemical liver function tests (LFTs), and computerized transaxial tomograms (CT scans) were used to evaluate abdominal and pelvic masses. Pretreatment serum carcinoembryonic antigen (CEA) was measured in 17 patients. Blood cell counts were obtained every 1–2 weeks; tumor measurements (on physical examination and chest roentgenogram), LFTs, and CEA every 3 weeks; and liver CT scans every 6 weeks.

AMSA was administered iv every 3 weeks over 30–45 minutes. The initial dose was 90 or 120 mg/m^2 except for one patient who received 60 mg/m^2 because of extensive prior chemotherapy and known limited marrow reserves. Subsequent doses were escalated by 30 mg/m^2 when the wbc count nadir was $>$ 3000/mm^3 or the platelet count nadir was $>$ 100,000/mm^3 following the previous dose. Doses were reduced by 30 mg/m^2 when the wbc count nadir was $<$ 2000/mm^3 or platelet count nadir was $<$ 75,000/mm^3. An adequate trial required two doses of AMSA or one dose producing myelotoxicity and a 3-week survival. Partial remission (PR) was defined as a $>$ 50% decrease in the summed products of the perpendicular diameters of all measurable lesions for $>$ 1 month; minor response was defined as a 25%–50% decrease in measurable lesions; stable disease was defined as a $<$ 25% change; and disease progression was defined as a $>$ 25% increase or the appearance of new lesions. KPS was used to evaluate subjective changes.

The median age of the 22 patients was 61 years (range, 46–80), and the median KPS was 60% (range, 50%–90%). Nineteen patients had received prior chemotherapy, with a median of two drugs (range, one to six); 14 had received prior irradiation to one or more sites and 12 had undergone partial or total resection of their primary tumor. All patients had objective, bidimensionally measurable metastatic tumor sites including lymph nodes or subcutaneous nodules in seven, pulmonary lesions in 11, malignant hepatomegaly in three, and abdominal/pelvic masses on CT scan in five. Twelve of 17 (70%) patients had elevated serum CEA ($>$ 5.0 ng/dl). Fourteen patients had more than one evaluable disease parameter for response evaluation.

RESULTS AND DISCUSSION

Two (9.5%; 95% confidence limits, 0%–22%) of 21 adequately treated patients achieved a PR for 3.5 and 2 months, respectively. Responses occurred in metastatic lung nodules in one patient and in multiple cutaneous

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TABLE 1.—Hematopoietic toxicity of AMSA in 21 patients

Dose (mg/m^2)	No. of courses	Wbc count nadir*		Platelet count nadir*	
		Median	Range	Median	Range
60	3	4.2	3.8–6.7	100	95–113
90	22	3.9	0.9–11.4	350	100–460
120	26	3.8	1.3–7.8	278	19–375
150	8	2.7	1.1–6.5	185	85–230
180	2	4.1	3.2–5.0	230	150–310

*Values $\times 10^3$ cells/mm^3.

nodules in the other. One patient is considered unevaluable for response, having died of rapidly progressive disease 11 days after the start of therapy. There were 61 courses of AMSA administered to 21 patients, with a median of three doses (range, one to six). Of 11 patients initially treated with 90 mg/m^2, five tolerated dose escalations to 120 mg/m^2. Of 11 patients given 120 mg/m^2, five tolerated dose escalations to 150 and 180 mg/m^2. Toxicity was dose-related (table 1) and consisted primarily of leukopenia, which occurred in 17 (81%) of 21 patients evaluable for toxicity. No episodes of myelosuppression-related sepsis or bleeding occurred. Other toxic effects included mild nausea and vomiting in four patients and alopecia in two.

AMSA demonstrated some antitumor activity in patients with metastatic bladder cancer who had received extensive prior chemotherapy. For these reasons, further studies, utilizing higher, more myelosuppressive doses, are indicated to more fully define AMSA's activity as a single agent, particularly in patients who have had no prior chemotherapy.

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Resistant Gestational Choriocarcinoma: Successful Treatment With Vinblastine, Bleomycin, and Cisplatin (VBP)[1,2]

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Two patients with resistant gestational trophoblastic neoplasms were treated with vinblastine, bleomycin, and cisplatin (Einhorn regimen). Both patients had failed multiple conventional therapeutic regimens including methotrexate, dactinomycin, high-dose methotrexate, and the modified Bagshawe regimen. Both patients achieved complete remissions and remain free of disease at 20 and 33 months following completion of therapy. The combination of vinblastine, bleomycin, and cisplatin is highly active in patients with resistant gestational trophoblastic neoplasms, and should be used as second-line therapy for poor-prognosis patients failing initial treatment. [Cancer Treat Rep 67:393–395, 1983]

Gestational trophoblastic neoplasms are the most sensitive of any human cancer to chemotherapy. This unique responsiveness is illustrated by the fact that only 18 of 326 (5.5%) patients treated between 1965 and 1979 at the New England Trophoblastic Disease Center died from metastatic cancer (1). The remainder of these patients achieved long-term complete remissions, often following the use of single-agent chemotherapy alone. Although these overall results are impressive, 15%–25% of patients have poor prognostic factors at diagnosis, and 25%–35% in this group ultimately die from their disease (1–3). We report two patients with gestational choriocarcinoma resistant to standard combination chemotherapy regimens, who achieved sustained complete remissions following therapy with vinblastine, bleomycin, and cisplatin (VBP).

CASE REPORTS

Patient 1

A 25-year-old female (Gravida 2, Para 0) presented to Vanderbilt Hospital in August 1980 after previous extensive treatment for metastatic gestational choriocarcinoma. Her past history included the evacuation of a hydatidiform mole by dilatation and curettage in March 1977. At that time, the β-human chorionic gonadotropin (β-HCG) was found to be 28,000 ng/ml. Her β-HCG levels had become negative by May 1977, when she was lost to follow-up.

In January 1980, she presented to another institution with right lower quadrant abdominal pain, a large pelvic mass, multiple pulmonary nodules, and a serum β-HCG of 28,000 ng/ml. Liver-spleen scan, brain computerized tomographic (CT) scan, and lumbar puncture were normal. She was treated initially with four cycles of methotrexate, dactinomycin, and chlorambucil (MAC) (4), followed by a fifth cycle containing dactinomycin alone. Her β-HCG value became briefly normal, but only 2 weeks later rose to 10.2 ng/ml. In March 1980, she received one cycle of the modified Bagshawe regimen (4), with no response (β-HCG rose to 26.8 ng/ml). In April 1980, total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed, with tumor found in the uterus and extending into the broad ligament.

Postoperatively, β-HCG values became negative but again increased 3 months later, and the patient was referred to Vanderbilt. A chest x-ray showed a new 1-cm right lung nodule. Serum β-HCG level was 20.4 ng/ml. She then received four cycles of chemotherapy with VBP as reported by Einhorn and Williams (4) (cisplatin, 20 mg/m^2 iv on Days 1–5, and vinblastine, 0.15 mg/kg iv on Days 1 and 2, every 3 weeks for four cycles; and bleomycin, 30 units iv on Day 2 and weekly for 12 doses).

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Following the first cycle, β-HCG level normalized (< 0.5 ng/ml), and it remained normal throughout subsequent therapy. The patient has now been followed for 20 months since completing chemotherapy, with normal β-HCG levels and no evidence of recurrent disease.

Patient 2

A 26-year-old female (Gravida 1, Para 0) presented to Vanderbilt Hospital in January 1979 with the recent onset of menometrorrhagia. Her past history included dilatation and curettage of a hydatidiform mole in October 1977, after which she was lost to follow-up. Evaluation at Vanderbilt Hospital revealed an 8-week-size uterus, a right adnexal mass, and a serum β-HCG of 2800 ng/ml. A laparotomy was performed, and a 3 × 3 × 3.5-cm mass was found in the uterine fundus extending into the right parametrium. Biopsy of this lesion was diagnostic of choriocarcinoma. Liver-spleen scan, abdominal CT scan, lumbar puncture, and head CT scan were normal.

The patient initially received two courses of methotrexate (1.0 mg/kg im, Days 1, 3, 5, and 7) with leucovorin rescue (0.1 mg/kg im, Days 2, 4, 6, and 8); and the β-HCG value decreased to 150 ng/ml but then increased. Two doses of high-dose methotrexate (6 g/m^2) at weekly intervals followed by leucovorin rescue produced no resposne. She then received three courses of dactinomycin (12 μg/kg/day for 5 days), but her β-HCG remained elevated at 115 ng/ml. Because of continued heavy vaginal bleeding, she underwent a total abdominal hysterectomy and right salpingo-oophorectomy in July 1979; viable choriocarcinoma was found involving the uterus and the right parametrium.

Following surgery her β-HCG levels rose from 200 to 500 ng/ml; she was then started on VBP therapy and received three cycles of this treatment, using 75% of the usual vinblastine dosage because of the extensive prior chemotherapy. After the first cycle, her β-HCG level had dropped to 5.7 ng/ml, and after three cycles it was 2.0 ng/ml. She had markedly elevated follicle-stimulating and luteinizing hormones, and following the administration of estrogen, her β-HCG level fell to < 0.5 ng/ml.

She has now been followed for 33 months, with constantly normal β-HCG levels and no evidence of recurrent choriocarcinoma.

DISCUSSION

Our two patients were clinically similar, and both had several poor prognostic features. Both patients had remarkably long intervals (15 and 33 months) between the treatment of a molar pregnancy and the diagnosis of trophoblastic malignancy. Both failed multiple chemotherapy regimens and had progressive disease at the time of initiation of therapy with VBP. Treatment with VBP produced rapid decline in β-HCG levels in both patients, with the women achieving normal β-HCG levels after one and three cycles, respectively.

Resistant trophoblastic neoplasms are rare, and information concerning treatment is limited to scattered case reports and small series. Second-line treatment has generally been much more difficult than has initial therapy. MAC has been the combination most often used in patients failing sequential single-agent therapy. Success with this approach has been limited, with only eight of 28 (18%) patients reported in five series achieving a complete response (5,6–8). Although Surwit et al (9) have reported complete remission in five of six poor-prognosis patients treated with the modified Bagshawe regimen (CHAMOCA) after failing initial therapy with MAC, these results could not be duplicated by Lurain et al (8), who had no complete responses among eight previously treated patients receiving CHAMOCA.

VBP has been highly successful in the treatment of testicular germ cell neoplasms (10,11), and also in the treatment of ovarian germ cell tumors (12,13). For this reason, these drugs have been sporadically used in patients with resistant gestational trophoblasic neoplasms, and several case reports have documented their activity. Two patients have received the VAB-III regimen (vinblastine, dactinomycin, cyclophosphamide, bleomycin, and cisplatin), and both achieved partial responses (13,14). Surwit et al (3), using lower doses of vinblastine, bleomycin, and cisplatin, have reported two patients who achieved complete remission, but no long-term follow-up has been published. Finally, Schlaerth et al (15) reported one patient with resistant gestational choriocarcinoma who achieved a sustained complete remission (24+ months) following treatment with VBP in dosages used in our two patients.

We believe that the achievement of complete remissions in both of the patients with resistant gestational choriocarcinoma seen recently at this institution is noteworthy. Although this regimen is associated with greater toxicity than are the other regimens used to treat trophoblastic neoplasms, side effects in large numbers of patients with testicular cancer have been manageable when treatment is given by experienced oncologists. The VBP regimen is also attractive in this situation because it contains three chemotherapeutic agents not used in first-line therapy for gestational trophoblastic neoplasms. For this reason, VBP is a more logical second-line choice for patients failing either sequential methotrexate-dactinomycin or MAC than is the modified Bagshawe regimen.

An aggressive approach to the unusual patient who fails standard regimens for gestational trophoblastic neoplasia is warranted. Long-term remissions and probable cures are achievable in these patients with the use of VBP. With the addition of VBP as a highly active

second-line regimen, it is likely that the already excellent treatment results for this malignancy can be even further improved.

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Phase II Study of Etoposide in the Treatment of Esophageal Carcinoma[1,2]

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Etoposide, in phase I studies, is a semisynthetic derivative of podophyllotoxin that showed encouraging activity in epidermoid carcinoma of the esophagus (ECE). Two of nine patients achieved a partial response (1). Because of these findings, a phase II trial of etoposide in the treatment of ECE was initiated at our institution.

Twenty-three patients with histologically proven ECE were entered in this study (patient characteristics and results are summarized in table 1). Criteria for entry into the study included measurable or evaluable disease, total wbc count $\geq 4000/mm^3$, platelet count $\geq 120,000/mm^3$, serum creatinine and serum bilirubin each ≤ 2 mg/dl, no therapy for 4 weeks prior to entry, anticipated survival ≥ 6 weeks, and informed consent.

Etoposide was administered as a slow iv infusion (45 mins) on Days 1, 3, and 5 of each 21-day cycle. Patients with Karnofsky performance scores (KPS) ≥ 70 were given a 120-mg/m^2 dose (360 mg/m^2/cycle), while patients with KPS ≤ 70 were given a 100-mg/m^2 dose (300 mg/m^2/cycle). Subsequent cycles were reduced by 60 mg/m^2 if the wbc count nadir was $< 3000/mm^3$ or the platelet count nadir was $< 100,000/mm^3$. In the absence of myelosuppression, subsequent cycles were escalated by 60 mg/m^2.

Of 23 patients entered, three were considered inevaluable for response because of early (≤ 2 wks) disease-related deaths. Fourteen evaluable patients had received prior chemotherapy, most with a combination of vindesine, cisplatin, and bleomycin (2). Six evaluable patients had not received prior chemotherapy.

Toxicity was primarily hematologic and occurred usually within 10–21 days of the first dose. Two patients died of pneumonia during the wbc count nadir. Alopecia was frequently observed. Nausea and vomiting were uncommon.

No complete or partial antitumor responses were seen in this trial. Two of six patients who had not received prior chemotherapy experienced brief minor responses.

TABLE 1.—Pretreatment characteristics and study results

	No. of patients
Entered/evaluable	23/20
Median age in yrs (range): 57 (45–80)	
Median KPS (range): 70 (40–80)	
Prior therapy	
Surgery	15
Radiation therapy	15
Chemotherapy	17
Disease sites	
Lymph nodes	10
Lung	9
Esophagus only	6
Hepatic	4
Mediastinal (by computerized tomographic scan)	3
No. of cycles of therapy/patient ≥ 2	4
Median (range): 1 (1–3)	
Myelotoxicity	
Median wbc count nadir (range): 2200 cells/mm^3 (0–7000)	
Median platelet count nadir (range): 133,000 cells/mm^3 (18,000–468,000)	
Complete/partial responses	0
Disease stabilization/minor response	1/3
Disease progression	16
Median survival in wks (range): 7 (2–47)	

Among 14 previously treated patients there was one patient with a brief minor response and one with stable disease for 2 months.

These findings preclude a 15% response frequency (with 95% confidence) in this population with ECE.

Although this negative result may be related to the extent of prior therapy in these patients, vindesine and methyl-GAG were identified as active agents in trials

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involving similar low KPS and pretreated patient populations (3,4).

We conclude that etoposide in the dose and schedule employed is inactive in patients with advanced ECE.

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Evaluation of Single-Agent Cisplatin in the Management of Non-Small Cell Carcinoma of the Lung: A Southwest Oncology Group Study[1,2]

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There has been encouraging prior experience with an outpatient cisplatin regimen of 50 mg/m^2 on Days 1 and 8 every 28 days in epidermoid tumors of the head and neck region (1) and of the esophagus (2). Because of this, the Southwest Oncology Group tested this regimen in patients with extensive non-oat cell lung cancer.

One hundred and eighty-one patients from 30 institutions were entered. Due to competing protocols, only 29 patients had pulmonary adenocarcinomas (27 fully evaluable), 21 had large cell undifferentiated carcinomas (13 fully evaluable), and 131 had epidermoid tumors (105 fully evaluable). For the adenocarcinomas and epidermoid tumors, an assessment of degree of differentiation was made by institutional pathologists. Partial response was defined as a 50% regression lasting at least 30 days.

No patient achieved a complete response. Overall, a partial response was achieved by 19 patients (10% of the eligible patients and 13% of the fully evaluable patients). This included 15 patients with squamous cell carcinoma (11% of the eligible patients) and four with adenocarcinoma (14% of the eligible patients). There were no responses in patients with large cell undifferentiated tumors. Twenty percent of all patient entries and 16% of responders had had prior chemotherapy.

Table 1 shows the impact of degree of differentiation on response rate and survival. Those patients with indeterminate differentiation are excluded. The data suggest that degree of differentiation may be an important factor in chemotherapy outcome.

Using standard Southwest Oncology Group criteria, the incidence of severe or greater toxicity was as fol-

TABLE 1.—Response rates and survival durations by histologic grade

	Response rate (%)	*P* value	Median survival (wks)	*P* value
Overall				
Well-differentiated	4	0.182	30	0.016
Poorly differentiated	14		21	
Squamous cell carcinoma				
Well-differentiated	5	0.202	28	0.09
Poorly differentiated	16		22	
Adenocarcinoma				
Well-differentiated	0	0.478	21	0.524
Poorly differentiated	18		21	

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lows: emesis, 14.3%; nephrotoxic effects, 2.2%; leukopenia, 1.7%; and thrombocytopenia, 1.1%. The only life-threatening toxic effect was leukopenia, with a total wbc count $< 1000/mm^3$ in two patients. There were no fatal toxic effects.

Cisplatin is widely used today as part of various combinations to treat non-oat cell lung cancer (3,4). Nevertheless, as other studies report (5), and as this study confirms in large numbers of patients, the effectiveness of cisplatin as a single agent to treat these tumors is quite modest.

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Phase II Study of Maytansine in Advanced Sarcomas[1,2]

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Fifteen patients with histologically confirmed, unresectable metastatic sarcomas were treated by rapid iv infusion with maytansine (0.6 mg/m^2) daily for 3 consecutive days every 3 weeks (1). Requirements for admission to the study included the presence of evaluable disease; expected survival of at least 2 months; at least semiambulatory performance status; and adequate renal, hepatic, and bone marrow functions. Four of the patients had received previous chemotherapy (table 1).

Following the initiation of treatment, maytansine doses

TABLE 1.—Patient characteristics and treatment results

Age (yrs)	Sex	Performance status*	Histologic diagnosis	Previous chemotherapy†	Response to maytansine	Toxic effects
59	M	2	Leiomyosarcoma, retroperitoneal	None	Disease progression	Diarrhea
75	M	0	Hemangiosarcoma, scalp	None	Stable disease	Diarrhea, peripheral neuropathy
58	M	3	Leiomyosarcoma, stomach	None	Stable disease	None
67	M	3	Leiomyosarcoma, ileum	None	Disease progression	Not evaluable
75	F	1	Malignant fibrous histiocytoma, breast	None	Disease progression	Nausea, diarrhea, alopecia
58	F	1	Lymphangiosarcoma, arm	None	Disease progression	None
78	M	1	Rhabdomyosarcoma, nose	None	Improvement	Nausea and vomiting, diarrhea
64	M	2	Leiomyosarcoma, jejunum	None	Stable disease	Nausea, diarrhea, alopecia, adynamic ileus
55	F	3	Malignant fibrous histiocytoma, paravertebral	None	Stable disease	Diarrhea
32	F	2	Osteosarcoma, ethmoid	HDMTX + VCR	Stable disease	Nausea and vomiting, diarrhea
33	F	1	Malignant fibrous histiocytoma, arm	None	Disease progression	Paresthesias
17	F	0	Malignant mesenchymoma, thigh	VCR, CYCLO, DACT, DOX, DTIC	Disease progression	None
73	M	2	Osteosarcoma, scapula	None	Disease progression	Diarrhea
16	M	0	Osteosarcoma, humerus	HDMTX + VCR	Disease progression	None
51	F	1	Osteosarcoma, vertebral	HDMTX + VCR	Stable disease	Diarrhea

*Eastern Cooperative Oncology Group criteria.

†HDMTX = high-dose methotrexate, VCR = vincristine, CYCLO = cyclophosphamide, DACT = dactinomycin, DOX = doxorubicin, and DTIC = dacarbazine.

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were modified for any excessive toxicity: hematologic, mucosal, neurologic, hepatic, or gastrointestinal.

None of the 15 patients experienced objective tumor regression, and only one patient experienced even a transient minor degree of tumor shrinkage. Six patients, however, did have stable disease for at least 6 weeks, with the longest continuing for 21 weeks. Eight patients experienced immediate disease progression following the initial course. Only four of the 14 patients for whom any toxic effects were adequately described experienced no toxicity. No patient was removed from the study because of drug toxicity, and no maytansine-related deaths occurred. Median survival for the 15 patients who received maytansine was 117 days.

Our data confirm the negative results of Wasserman (2) and those of Borden et al (3) using weekly doses of 0.5–0.75 mg/m^2 and once every 3 week doses of 1.5 mg/m^2, respectively. Thus, maytansine is not active against advanced sarcomas.

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Mitoxantrone for Treatment of Patients With Refractory Small Cell Carcinoma of the Lung: A Southwest Oncology Group Study[1,2]

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This phase II study was performed to evaluate the therapeutic efficacy of the anthracene derivative mitoxantrone in patients with histologically confirmed small cell carcinoma of the lung who have progressive disease after conventional therapy. Eligibility criteria included a lesion measurable in two dimensions (preferably outside an area of prior radiotherapy), an anticipated survival of at least 8 weeks, a performance status of ≤ 3 (Southwest Oncology Group criteria), and recovery from toxic effects due to previous therapy. Patients with and without prior treatment with doxorubicin were included in the study to determine if treatment with that drug conferred increased clinical resistance to mitoxantrone.

Sixty-five patients were entered in the study. Nine patients had early deaths due to progressive tumor (ie, within 3 weeks of receiving the drug), and two refused further therapy with mitoxantrone after one dose of the drug. Table 1 details the characteristics of the 54 evaluable patients. Of note is that 13 evaluable patients had received no prior doxorubicin.

Mitoxantrone was administered at an initial dose of 12 mg/m^2 as an iv infusion over 30 minutes in 150 ml of 5% dextrose in water. The dose was repeated every 3 weeks. The dose and schedule were determined to be safe based on an initial phase I trial (1). Doses were escalated or de-escalated by 2-mg/m^2 increments based on nadir wbc counts. Therapy was continued until evidence of disease progression. Only one patient was able to receive an escalated dose of 14 mg/m^2. One patient required de-escalation to a dose of 10 mg/m^2. There was one partial response in the 41 evaluable patients with prior doxorubicin exposure (2% response rate) and one partial response in 13 evaluable patients without prior doxorubicin exposure (8% partial response rate). The partial responses lasted 1$^1/_2$ and 5$^1/_2$ months, respectively.

TABLE 1.—Characteristics of patients studied

Characteristic	No. of patients
Entered	65
Evaluable	54
Age in yrs	
Median: 58	
Range: 37–80	
Sex	
Male	34
Female	20
Performance status*	
Median: 2	
Range: 0–3	
Prior doxorubicin	
Yes	41
No	13
Prior chemotherapy	
1–4 drugs	22
5–9 drugs	32
Prior radiation therapy	
Yes	40
No	14
No. of cycles of mitoxantrone therapy	
Median: 2	
Range: 1–6	
Response	
Complete	0
Partial	2
Mixed	2
Progression	50

*Southwest Oncology Group criteria.

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Toxicity data based on 87 courses of therapy showed the major toxic effect of mitoxantrone to be leukopenia. The median total wbc count for patients was 2000/μl (range, 400–13,700), with the median granulocyte count being 1100/μl (range, 144–10,686). Twelve patients had granulocyte counts < 1000/μl, and six of the 12 had granulocyte counts < 500/μl. The median Day of granulocyte nadir was Day 12, with full recovery by Day 21. No drug-related deaths occurred. Thrombocytopenia was not a problem with this drug dose; the median platelet count was 150,000/μl (range, 70,000–490,000). Seven patients developed platelet counts < 100,000/μl, and no patient developed a platelet count < 50,000/μl. Nonhematologic toxicity was mild and consisted of nausea and vomiting. There was no evidence of cardiac, renal, or hepatic toxicity. Cardiac ejection fraction studies were not consistently followed in this study, so subclinical cardiac effects of mitoxantrone could not be determined.

Unfortunately, despite a biologic effect of mitoxantrone on wbcs, there was only minimal (< 10%) antitumor activity noted in these patients with small cell lung cancer who had been heavily treated previously with other anticancer agents. Because of similarities between proposed mechanisms of action for mitoxantrone and doxorubicin, there could be cross-resistance between these two drugs. However, in this study there were no significant differences in response rates to mitoxantrone for patients who had and who had not received prior treatment with doxorubicin.

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Megestrol Acetate Flare

"Tamoxifen flare" has been well-described and is considered to be a harbinger of clinical response to tamoxifen in patients with metastatic breast carcinoma. It is usually manifested by markedly increased bone pain in patients with breast cancer metastatic to bone. The usual duration of pain is several days to, at most, several weeks. I am not aware of any previous reports of "megestrol acetate flare" in breast cancer patients. On March 2, 1982, a 62-year-old woman with extensive bone metastases from breast carcinoma started treatment with megestrol acetate (40 mg orally four times daily). Two days later severe diffuse bone pain was noted, particularly in the skull, and was accompanied by a fever of 38.3°C. In addition, a 3-cm mass at the angle of the right mandible was palpated. Roentgenogram of this area showed extensive lytic involvement of the mandible. After taking 15 tablets of megestrol (600 mg), the patient stopped the medication. By March 9, 1982, the pain was gone and megestrol acetate treatment was started at a dose of 40 mg daily. The dose was gradually increased to 40 mg four times daily. The bone pain has not recurred to date. Two palpable bony masses in the skull that, on skull roentgenogram, had been observed overlying lytic bone metastases have disappeared. The carcinoembryonic antigen level has decreased from 16.6 ng/ml on March 30, 1982, to 6.0 ng/ml on June 22, 1982. The patient is now asymptomatic. Because of the danger of fracture, the right mandible was irradiated; therefore, we could not use this lesion as a measure of response. This case suggests that a flare in bone pain may be a useful sign in predicting response to megestrol acetate.

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The Cytarabine Syndrome in an Adult

Cytarabine (Ara-C) is a chemotherapeutic agent widely used in the treatment of acute leukemia and non-Hodgkin's lymphoma. Major side effects include myelosuppression, nausea, vomiting, and mucositis. A syndrome of fever, conjunctival suffusion, and maculopapular rash has also been described in children who had received large cumulative doses of Ara-C (mean, 3200 mg/m^2) (1). This syndrome has not been well-described in adults receiving single-agent Ara-C. In this report we describe an adult with T-cell lymphoma, who had no previous exposure to Ara-C, but who experienced a reproducible adverse reaction to Ara-C resembling that described in children. It could be prevented by pretreatment with corticosteroids and did not occur when other chemotherapeutic agents were administered concomitantly with Ara-C.

CASE HISTORY

A 64-year-old white male was admitted for treatment of a diffuse large cell lymphoma involving lymph nodes and skin. His lymphoma was diagnosed in 1977 and had been treated with multiple chemotherapy regimens which included cyclophosphamide, vincristine, procarbazine, prednisone, bleomycin, and doxorubicin. Despite this therapy, he did not achieve a prolonged, complete remission. He was referred to the University of Massachusetts Medical Center for treatment in a prospective study of relapsed non-Hodgkin's lymphoma. Physical examination at that time revealed multiple cutaneous nodules and lymphadenopathy. Biopsy of the lymph node and skin nodules revealed a malignant lymphoma, large cell, noncleaved, which both by morphology and cell surface markers was consistent with a T-cell malignancy [$P_{23,\ 30}$, (+); monoclonal anti-T (+); calla (−); surface or cytoplasmic immunoglobulin (−)]. The patient then started receiving a 24-hour infusion of Ara-C (100 mg/m^2/24 hours); no other medications were being administered at that time. Within 12 hours after the Ara-C infusion, the patient developed a temperature > 39°C, confusion, and a diffuse maculopapular skin rash. These symptoms persisted throughout the Ara-C infusion. Cultures of blood, urine, and cerebrospinal fluid were negative for bacteria, fungi, and viruses. Spinal fluid analysis, cbc, and chest x-ray were all within normal limits. On the fourth day of the infusion, cor-

ticosteroid treatment (hydrocortisone, 40 mg every 6 hours) was initiated. This resulted in a rapid resolution of symptoms, despite continuation of Ara-C for another 24 hours. Both Ara-C and corticosteroids were discontinued, and the patient was discharged. Three weeks later the patient was again admitted for a second course of single-agent Ara-C. Initially, no steroids were given and the same reaction (fever, confusion, and rash) occurred within 24 hours after the initiation of therapy. Again, an extensive workup failed to reveal any other cause for these symptoms. Steroids were given on the third day and resulted in rapid resolution of the symptoms, despite continuation of the Ara-C infusion. Despite side effects, a third course of Ara-C was given because the patient had demonstrated a definite therapeutic response. With steroid premedication, there was no reaction to Ara-C. Several subsequent courses of Ara-C combined with cyclophosphamide and vincristine did not result in febrile reaction or rash. During these courses, the prednisone dose was decreased. Despite the elimination of prednisone, the patient had no adverse reaction. When Ara-C was resumed as a single agent 6 months later, prednisone therapy was again required to prevent a febrile reaction.

DISCUSSION

This case appears to represent an example of the Ara-C syndrome in an adult. Castleberry et al (1) reported six children who experienced a syndrome characterized by fever, malaise, joint or bone pain, maculopapular rash, and conjunctivitis while receiving single-agent Ara-C. Prior to the reaction, these patients had received chemotherapy for an average of 13.5 months and had been exposed to 2298–5387 mg/m^2 of Ara-C (mean, 320 mg/m^2). The authors postulated that a hypersensitivity reaction may have been involved. Several other reports of similar reactions have been described (2–4). Those reports were complicated by the simultaneous administration of several agents in addition to Ara-C. Rose and Bateman (3) successfully used hydrocortisone to prevent this reaction. Several authors have also reported similar reactions, predominantly fever, with single-agent "high-dose" Ara-C (5).

In our patient, there was a clear temporal association of the administration of single-agent Ara-C with the development of systemic symptoms. This reaction, in contrast with the report of Castleberry et al (1), occurred without previous exposure to Ara-C. This case also demonstrated that this reaction to Ara-C can be prevented by premedication with corticosteroids and does not necessitate discontinuation of an efficacious drug. While the precise pathophysiology of this syndrome remains unclear, a hypersensitivity reaction (Type 1) does seem to be a likely etiology. This possibility is supported by the ability of corticosteroids to suppress the reaction, and by the fact that simultaneous administration of other chemotherapeutic/immunosuppressive agents appeared to decrease the severity of the reaction. Other pathophysiologic mechanisms are certainly possible. It is also of interest that this patient had a lymphoid malignancy. The patients as described by Castleberry et al also had lymphoid malignancies (acute lymphocytic leukemia or lymphoma); however, cell surface markers were not described. The fact that the current patient had a T-cell lymphoma is of unknown significance.

It appears from available data that a clinically reproducible reaction to Ara-C, "the Ara-C syndrome," does exist even with "conventional" dose. There may be a higher incidence than previously appreciated. Physicians treating patients with Ara-C should be fully aware of this syndrome, which should be considered only after other causes of the associated symptoms are ruled out.

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Use of Dimethyl Sulfoxide in Limiting Tissue Damage Caused by Extravasation of Doxorubicin

Accidental subcutaneous doxorubicin extravasation may provoke a prolonged and severe cutaneous inflammatory reaction for which there is currently no simple and effective treatment method.

Following reports that topical dimethyl sulfoxide (DMSO) prevents doxorubicin-induced skin ulceration in rat and pig skin (1), we are reporting the successful use of DMSO in limiting tissue damage in patients following subcutaneous doxorubicin extravasation.

CASE REPORTS

Patient 1

A 49-year-old female receiving iv doxorubicin and cyclophosphamide for metastatic breast carcinoma experienced a 2–3-ml doxorubicin extravasation (2 mg/ml) into the medial aspect of the left cubital fossa. The infusion was discontinued immediately, 1–2 ml of fluid was withdrawn through the needle, and 5 ml of sodium bicarbonate (8.4%) was instilled. Ice packs were applied to the area for 24 hours.

Four days later an 11 × 6-cm area on the medial aspect of the cubital fossa was markedly edematous, erythematous, and indurated, limiting elbow extension by 50°. The patient required hospitalization for parenteral narcotic analgesia. DMSO (analytic grade; maximum limits of impurity of nonvolatile residue, 0.01%; water, 0.2%; Ajax Chemicals, Australia) was applied to the area daily for 14 days. Within 48 hours the pain had resolved. No ulceration occurred. Eight months later, the residuum is a 3 × 2.5-cm indurated area on the medial aspect of the cubital fossa, limiting elbow extension by 40°. There were no neurologic, vascular, or dermatologic sequelae.

Patient 2

A 53-year-old woman being treated with cyclophosphamide, doxorubicin, vincristine, and prednisone for diffuse histiocytic lymphoma had 3 ml of doxorubicin (2 mg/ml) extravasated into the lateral aspect of the right cubital fossa. One milliliter of fluid was withdrawn and 2 ml of sodium bicarbonate (8.4%) was injected. DMSO was painted onto the area, ice packs were applied, and the arm was elevated for 24 hours. DMSO was applied every 6 hours for 7 days and twice daily for a further 7 days. Two months later all that remains is a 5 × 6-cm pigmented area on the lateral aspect of the cubital fossa, with no functional or neurovascular impairment.

Patient 3

A 51-year-old male being treated with doxorubicin, cyclophosphamide, and vincristine for metastatic small cell carcinoma of the lung had a doxorubicin extravasation subcutaneously (2 mg/ml) onto the volar surface of the left wrist, causing a 4 × 5-cm edematous, erythematous area over the flexor tendons. The infusion was stopped and 2 ml of sodium bicarbonate (8.4%) was injected. DMSO was applied and the arm was elevated, with an ice pack over the area. DMSO applications were applied every 6 hours for the first week and twice daily for 1 additional week. One month later there is a 4 × 5-cm pigmented area over the flexor tendons but no functional impairment.

DISCUSSION

Despite careful technique, extravasations occur in up to 6% of antineoplastic drug infusions (2).

The major problem following doxorubicin extravasation is the continuing damage to adjacent and underlying normal tissues, even though only minor damage is initially apparent. Anthracycline levels can be measured in adjacent tissues for months after extravasation (3). Early wide surgical excision of necrotic and adjacent tissue has therefore been advocated (4).

Other suggested treatments including local instillation of steroids, sodium bicarbonate, and ice applications do not reliably prevent severe tissue damage. Because of this, following doxorubicin extravasation inadequately responding to these local measures (Patient 1) and wishing to avoid the problems of extensive surgery, we employed DMSO, based on the reported animal data. In rat and pig skin, topical DMSO prevented doxorubicin-induced ulceration which was not prevented by nine other agents including methylprednisolone and sodium bicarbonate (1).

In the reported cases we believe that the cutaneous application of DMSO significantly limited the extent of tissue injury from that previously experienced, particularly since the doxorubicin extravasation occurred in sites where underlying tendons and neurovascular structures were particularly at risk.

Minor skin irritation was the only toxic effect from DMSO and no extravasation site recall phenomena (5) occurred when doxorubicin was subsequently employed.

While recognizing the difficulty in confirming this result in a controlled clinical trial, we feel that DMSO is a useful and nontoxic agent in this potentially serious situation and requires further clinical evaluation.

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Influence of Pentagastrin Administration on Survival of Mice With L1210 Leukemia

There is an old concept that leukemia may be, in essence, a deficiency disease; the analogy has been made with pernicious anemia where the maturation of cells, as in some forms of leukemia, is interrupted (1,2). This idea has prompted us to investigate whether treatment with pentagastrin could modify the course of experimental leukemia.

DBA/2G mice with L1210 leukemia were supplied by the Cancer Institute in Gliwice, Poland. Thirty-five (DBA/2G × C57BL) F_1 hybrid mice were each inoculated ip with L1210 cells (2×10^6/0.2 ml). The L1210 cells were derived from peritoneal exudates of DBA/2G mice serving as hosts for passage of the leukemia. The animals were divided into one control group and three experimental groups. The experimental animals received daily ip injections of pentagastrin (Acignost VEB Berlin Chemie) beginning on Day 1 after leukemia transplantation. The injections were continued until death of the animal or, in the case of cure, until Day 30. Daily doses were: Group 1, 2 μg; Group 2, 10 μg; and Group 3, 20 μg.

Survival times of the untreated control group (all animals died on Day 9) and the experimental groups are shown in table 1. The best results were obtained in Group 1, ie, in mice that received the lowest dose of pentagastrin; 30 days following leukemia transplantation seven of ten animals were still alive and considered cured. Because the better survival rate in animals given the lowest dose was a somewhat unexpected result, the experiment was repeated twice with the same 2-μg dose and under the same test conditions; similar results were obtained.

This report demonstrates that pentagastrin alone in the treatment of L1210 leukemia in mice results in a considerable prolongation of survival or a cure. At present we are unable to give a satisfactory explanation of the good results with pentagastrin administration in the treatment of L1210 leukemia.

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Table 1.—Survival of L1210 leukemia-bearing DBA/2G × C57BL mice treated with pentagastrin

Group No.	Pentagastrin dose (μg/day)	No. of animals	Survival (days)*							
			8	9	10	12	20	23	27	≥ 30†
Control	—	5	—	5	—	—	—	—	—	—
1	2	10	—	1	1	1	—	—	—	7
2	10	10	1	2	2	—	1	—	—	4
3	20	10	2	1	2	—	—	1	1	3

*Values = No. of mice.
†Cured.

The extravasation of some iv administered chemotherapeutic agents can lead to a severe, local sclerosing reaction. The sequelae of this process are often restriction of limb mobility and/or the necessity for skin engraftment. Doxorubicin (ADR) and dactinomycin are best known for this side effect. We have designed an experiment by which new drugs can be easily tested for their potential to cause extravasation injury.

A modification of the rat model described by Rudolph et al (1) and more recently by Svingen et al (2) was used. Sprague-Dawley rats (250 g) were anesthetized with pentobarbital (60 mg/kg) and both flanks were shaved. As a control, all rats were injected on one side above the dermis (as described in ref 2) with 0.5 ml of a 0.9% saline solution containing 1 mg of ADR. The drug to be tested was injected in a similar manner on the opposite side.

When the test was positive (ADR side in 19 of 23 rats), the typical picture of erythema developed in 2–3 days, leading to ulceration by Days 4–7; healing occurred by Day 12. The healing eschar was measured on Day 12 in its largest dimension and was scored as follows: 1+ = 0–5 mm; 2+ = 6–10 mm; and 3+ = > 10 mm. Scores were added and expressed as a response fraction of the possible total cumulative score of 3+ for each rat. Results are given in table 1.

These data confirm the ulcerogenic propensity of ADR and dactinomycin and suggest that bisantrene has a relatively high potential in this regard. Aclarubicin, on the other hand, is comparatively weaker. Our model for testing the vesicant action of new drugs has an advantage over other models [such as the mouse model (3)] in that relatively large and inexpensive animals are used. Disadvantages include use of pentobarbital as an anesthetic which might possibly alter tissue response (4).

We conclude that all new drugs should be screened for vesicant activity before introduction into the clinical setting. We have presented one simple model for this purpose but others are available. A wide range of dosages and concentrations should be included in such screening.

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Table 1.—Test results

Drug	Dose (mg)	No. of rats	Response fraction
ADR	1.0	23	0.62
Dactinomycin	0.5	5	0.80
Bisantrene	1.0	5	0.46
Aclarubicin	0.5	4	0.16
Aclarubicin	1.0	4	0.00
Aclarubicin	2.0	5	0.13

Second Malignancy After Cisplatin, Vinblastine, and Bleomycin (PVB) Chemotherapy: A Case Report

We wish to report the case of a man treated with combination cisplatin, vinblastine, and bleomycin (PVB) chemotherapy, who later presented with florid stage II bladder cancer.

The patient, a 26-year-old white man employed as a refuse collector, underwent right radical orchiectomy for a teratocarcinoma in September 1977. Postoperative staging investigations were normal, and he was therefore considered stage I; no further therapy was given. Six months later a rising level of alpha-fetoprotein was noted. Chest x-ray, lymphangiography, abdominal ultrasound, and blind scalene node biopsy were within normal limits, and the patient underwent exploratory laparotomy. Two tumors were apparent in the paraaortic region, and both were resected. Postoperative alpha-fetoprotein levels fell to within the normal range.

In view of the fact that a formal retroperitoneal node dissection was not performed, three cycles of adjuvant modified PVB chemotherapy were given at 21-day intervals from July to October 1978. Cisplatin was given at a dose of 80 mg/m^2 on Day 5, vinblastine at a dose of 0.2 mg/kg on Days 1 and 2, and bleomycin as a 24-hour infusion on Days 1–5 at a dose of 15 mg/m^2. Following this therapy, maintenance vinblastine was given at a dose of 0.15 mg/kg iv monthly for 12 months. Chemotherapy was completed in December 1979.

The patient remained well, with normal follow-up x-rays and serum markers until August 1982 (59 months after first presentation and 32 months after completing maintenance chemotherapy). He then presented with a 2-week history of profuse hematuria. Iv urography revealed bilateral hydronephrosis with a pelvic mass. At cystoscopy the bladder was replaced by an extensive papillary tumor which on bimanual examination was palpable. Histologically this proved to be a grade 2 transitional cell carcinoma. The patient subsequently underwent total cystectomy, with fashioning of an ileal conduit. The tumor was found to have infiltrated the muscularis mucosa, but adjacent iliac lymph nodes sampled at the time of cystectomy showed no evidence of tumor spread ($T_2N_0M_0$). Postoperative recovery was uneventful, and the patient continues, with regular follow-up, to be without evidence of recurrent disease.

The occurrence of two primary tumors within a 5-year period in this young man is remarkable. There is no recognized association between germ cell tumors of the testis and bladder cancer. A literature search has revealed only one similar case of second malignancy (1): acute lymphocytic leukemia occurring after both radiotherapy and PVB chemotherapy were given for a mediastinal germ cell tumor.

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FOCA Oncology Fellowships

Brussels, Belgium

Applications are now being accepted for 1983–1984 Fondation pour la Chimiothérapie Anticancéreuse (FOCA) fellowships with the Cancer Center of the University of Brussels. The fellowships are geared toward medical oncologists from the United States who are board-certified in oncology or have equivalent training and want to work one year or longer as a staff member in the Department of Medicine and Clinical Investigation of the Institut Jules Bordet, The Cancer Center of the University of Brussels. The chief of the department is Dr. Jean Klastersky.

Stipend is approximately $35,000–$45,000 (2 million Belgian francs) depending on the exchange rate.

For further information, contact: Professor Henri Tagnon, President of FOCA, Institut Jules Bordet, 1, Rue Héger-Bordet, B-1000 Bruxelles, Belgium (telephone: 02/538.27.66, Telex: 22 773).

Nutrition and Cancer

Los Angeles, California, June 8, 1983

This symposium, sponsored by the Hospital of the Good Samaritan, will be held at the Biltmore Hotel. The fee, including the luncheon, will be $80 for practicing physicians, $45 for allied health professionals, and $35 for interns, residents, fellows, and retired physicians. This symposium meets the criteria for 6 hours of Category 1 Credit toward CMA and AMA certificates in Continuing Medical Education.

For more information, contact: Bonnie VanWaardenburg, The Hospital of the Good Samaritan, 616 S Witmer St, Los Angeles, CA 90017 (telephone: 213-977-2345).

Neuro-Oncology and Neuropathology Fellowships

San Francisco, California, July 1983 and 1984

The University of California, an equal opportunity/affirmative action employer, is sponsoring these fellowships. The neuro-oncology fellowship emphasizes clinical training in adult and childhood primary tumor therapeutics and research training in the Brain Tumor Research Center laboratories.

The neuropathology fellowship emphasizes primary tumor pathology and offers research opportunities in the Brain Tumor Research Center and the Department of Pathology.

For more information, contact: Victor Levin, MD, Brain Tumor Research Center, HSW-783, University of California, San Francisco, CA 94143 (telephone: 415-666-2905).

International Symposium on Detection and Treatment of Minimal Residual Disease in Acute Leukemia

Rotterdam, The Netherlands, October 12–14, 1983

This symposium, co-sponsored by the Comprehensive Cancer Center Rotterdam, focuses on detection and treatment of subclinical disease in acute leukemia. The relative value of chemotherapy and bone marrow transplantation programs as well as the usefulness of new experimental approaches (cell separation, cell culture systems, monoclonal antibodies) and their integration into clinical management will be the major topics. International experts in the field will participate in specific panel discussions on selected subjects. Abstract forms must be received *before July 1, 1983.*

For further information, contact: Dr. J. W. van der Velden, % Organization Committee, Leukemia Symposium "Detection and Treatment of Minimal Residual Disease in Acute Leukemia", Dr. Daniël den Hoed Kliniek/Rotterdam Radio Therapeutic Institute, P. O. Box 5201, 3008 AE Rotterdam, The Netherlands.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

Cincinnati Cancer Conference: Gastro Intestinal Malignancies

Cincinnati, Ohio, November 4–5, 1983

This conference is sponsored by the Bethesda Hospital and Deaconess Association, and the University of Cincinnati.

For further information, contact: Thomas O'Connor, Medical Staff Education, Bethesda Hospital, 619 Oak St, Cincinnati, OH 45206.

International Symposium of the Ludwig Boltzmann Research-Institute for Infections and Tumors of the Urinary Tract

Vienna, Austria, November 25–26, 1983

Symposia topics include: predictability of cancer chemotherapy with special respect to renal, bladder, prostatic, and testicular tumors; persistent asymptomatic microhematuria; and percutaneous surgery of the kidney—diagnostic and therapeutic approach—indication, techniques, risks, and results.

For further information, contact: Dr. P. Porpaczy, % Wiener Medizinische Akademie, Alser Straße 4, A-1090, Vienna, Austria. Telephone: (0222) 42 71 65, Cables: Medacad Wien.

Fourth Conference on Human Tumor Cloning

Tucson, Arizona, January 8–10, 1984

This conference will be held at the University of Arizona Cancer Center, and a combination of invited and competitively selected papers, demonstrations, and posters will be presented. Deadline for submission of abstracts is October 1, 1983.

Dr. Sydney E. Salmon and Dr. Jeffrey Trent are the co-chairmen for the event.

For more information, contact: Mary Humphrey, Conference Coordinator, University of Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-6044).

Fourth International Conference on the Adjuvant Therapy of Cancer

Tucson, Arizona, March 21–24, 1984

This conference will be held at the Tucson Convention Center under the sponsorship of the University of Arizona Cancer Center. Drs. Stephen E. Jones and Sydney E. Salmon will serve as conference co-chairmen. The deadline for submission of abstracts, prepared in the AACR/ASCO format, is *November 1, 1983*.

For further information, contact: Mary Humphrey, Conference Coordinator, University of Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-6044).

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